TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS

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IN

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BY

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Dedicated

to

my family

&

Teachers

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Dr Bableen Kaur Ghotra

ABBREVIATIONS

AG	Aktiengesellschaft (joint-stock company)
AHG	Antihuman Globulin
AIHA	Autoimmune Hemolytic Anemia
CAT	Column Agglutination Technology
DAT	Direct Agglutination Test
DCT	Direct Coombs' Test
DTT	Di Thio Threitol
FDA	Food and Drug Administration
HDFN	Hemolytic Disease of Fetus and Newborn
HEK	Human Embryonic Kidney
HTR	Hemolytic Transfusion Reaction
H/O	History Of
HPC	Hematopoietic Progenitor Cell
IAT	Indirect Agglutination Test
ICT	Indirect Coombs' Test
ID-MTS	ID-Micro Typing System
Ig	Immunoglobulin
IHBT	Immunohematology and Blood Transfusion
IRL	Immunohematology Reference Laboratories
ISBT	International Society of Blood Transfusion

IVIG	Intravenous Immunoglobulin
Mf	Mixed field
No.	Number
PEG	Polyethylene Glycol
PRBC	Packed Red Blood Cell
RBC	Red Blood Cell
RCA	Red Cell Alloimmunization
RDP	Random Donor Platelets
Rh	Rhesus (Rhesus monkey)
RSA	Road side Accident
SCD	Sickle Cell Disease
SD	Standard Deviation
SDP	Single Donor Platelets
SPRCA	Solid Phase Red Cell Adherence Assay
WNL	Within normal limit.

ABSTRACT

Introduction: Providing safe blood includes both testing for infectious marker and protection from deleterious sequelae like hemolytic transfusion reactions resulting from allo-immunization against red cell antigen. Ever increasing efforts are put into procuring safe blood which leads to incorporation of regular screening protocols for detection of unexpected immune antibodies at various centers across the globe.

Aim of Study: The present study aims to screen antibodies and its identification in blood donors and to provide blood that lacks corresponding antigen to the patient.

Materials & Methods: This prospective study was done on all the Whole blood donor samples at our Tertiary care centre. Blood Samples were screened for irregular red cell antibodies from January - December 2019. Antibody screening was performed by solid phase antibody detection method using Capture–R ready screen (pooled cells) and positive samples were further confirmed using 3 cell and 11 cell panel.

Results: A total number of 26772 whole blood donors donated at our Blood Centre during the study. Among them, 79(0.3%) were found positive for antibody screen performed via solid phase antibody detection method using Capture–R ready screen (pooled cells). Donors within the age group of 20–30 years showed maximum number (n =34; 43%) of antibody screen positivity. The percentage of positive screen among donors gender-wise was 75/26129 (0.28%) males and 4/643 (0.62%) females. The results showed statistically a higher prevalence of RBC allo-antibodies in females than males. On identification among 79 cases, 48 (61%) had allo-antibodies and 4(5.1%) had auto-antibodies (warm auto antibodies) while 27(34.2%) were inconclusive. Anti-M (n =28; 35.4%) was the most common antibody identified, followed by anti-P1 (n = 5; 6.3%) plus one Anti-P in combination with Anti-Le^b.

Clinical Significance: Antibodies against red cells can be found in blood donors, detection of which adds a layer of safety in transfusion.

Keywords: Antibody, Antigen, Alloantibody identification, alloantibody screening, donor screening, HTR.

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INTRODUCTION

Karl Landsteiner discovered the human blood group system in 1901 [1] and from there on boom in the field of blood transfusion came to be. Even after more than 100 years, a lot needs to be understood and discovered in this field. Our knowledge is quite limited and much remains to be explored. Immunoglobulins, or antibodies, have special significance for Transfusion Medicine, because antigens present on transfused cells may cause reactions in the recipient and complicate therapy.

A major portion of testing is focused on the prevention, detection, and identification of blood group antibodies and on the typing of RBC antigens in transfusion services. A red cell antigen phenotype and/or genotype is detected prior to transfusion with the aim of identifying absent red cell antigens in order to provide antigen-negative red cells for transfusion. Antigens that may cause allo-immunization are: D, E, e, C, c, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s, M, N, Le^a, and Le^b. It's done as soon as possible before the first transfusion.

Antibody detection methods are focused more on “irregular" or “unexpected” than the “expected” antibodies of the ABO system. Red cell antibody anti-A and anti-B are the naturally occurring antibodies that are found in the human serum. Unexpected antibodies are the immune alloantibodies of primary interest in this study, which are produced in response to red blood cell (RBC) stimulation through transfusion, transplantation, or pregnancy. Some unexpected antibodies may be “naturally occurring” that are produced without RBC stimulation. Naturally occurring antibodies may form as a result of exposure to environmental sources, such as pollen, fungus, and bacteria, which have structures similar to some RBC antigens.

Antibodies produced in one individual are transmitted to another via plasma-containing blood components or derivatives e.g. intravenous immunoglobulin (IVIG) are known as passively acquired antibodies, a third category of unexpected antibody. The presence of naturally occurring and passive antibodies may complicate the detection and

identification of immune, clinically significant antibodies. Clinically significant antibodies are those that cause decreased survival of RBCs possessing the target antigen. These antibodies are typically IgG antibodies that react at 37°C or in the antihuman globulin (AHG) phase of the indirect anti-globulin test.

Irregular red cell antibody is of 2 types: allo-antibody and auto-antibody. Alloantibody is produced against the antigen that is lacking, whereas autoantibody is produced to an antigen that is present. Auto-antibodies may also complicate the detection of clinically significant antibodies because they react with all RBCs tested, masking the presence of clinically significant allo-antibodies. After detection, an antibody identification panel is performed to determine the specificity of the antibody (or antibodies) present. Once identified, the antibody's clinical significance can be ascertained and the appropriate transfusion considerations put into place.

The AABB's Standards for Blood Banks and Transfusion Services requires the use of an antibody screen to detect clinically significant antibodies in both the blood donor and the intended recipient as part of pre-transfusion compatibility testing.

The donors may be used as a source of antigen-typing sera and antigen-negative RBC units. An antibody screen may be included when evaluating the compatibility of hematopoietic progenitor cell (HPC) and bone marrow donors with the intended transplant recipient. Antibodies detected in these donors may indicate that additional processing steps are required to reduce the plasma in the product. An antibody screen is included in standard prenatal testing for obstetric patients to evaluate the risk of HDFN in the fetus and to assess the mother's candidacy for Rh-immune globulin (RHIG) prophylaxis.

Guidelines for the screening of donated blood for the presence of irregular red cell antibodies have been laid down by The National Blood Policy, India, 2007. The incidence of transfusion reactions due to irregular red cell antibodies in donor blood is rare scenario.[2] However; the presence of such antibodies can occasionally cause severe transfusion reactions if a large amount of plasma or whole blood is

transfused as in the cases of massive transfusions or in pediatric population.

Procurement of safe blood for transfusion includes not just testing for infectious markers but also protection from Hemolytic Transfusion Reaction (HTR) resulting from allo-immunization. Ever increasing efforts at improving blood safety has led to incorporation of Regular Screening Protocol for detection of Unexpected Antibody.

AIMS AND OBJECTIVES

The present study aims:

- To detect the antibody and its Identification in Blood Donors.
- To provide blood that lacks the corresponding antigen to the patient.

REVIEW OF LITERATURE

In this study, we are dealing with Red cell antigens and their associated unexpected allo-antibodies; go over a bit about autoantibodies, methods of testing blood sample for antibodies, literature involving some techniques for ICT, DCT, Antibody detection, screening and identification. Some cases of persistence of alloantibodies for a certain time period will be seen and methods taken to ensure safe blood preventing alloimmunization via good cross matched blood for transfusion.

HISTORICAL ASPECTS OF SCREENING METHODS

INDIRECT COOMBS TEST (ICT) for Antibody Detection

One of the initial tests performed on the serum/plasma of blood samples of healthy donors and it has a history of more than 70 years to how it has reached till today. In the year 1945, in The British Journal of Experimental Pathology there was publication mentioning a new test, it described for the detection of incomplete or weak antibodies, with special reference to Rh antibodies.[3] The principle of test is based on the use of an antihuman-globulin serum which will agglutinate red cells that have been sensitized, and thus have globulin antibody adsorbed at some points on their surface. Later the principle of this test was applied to a number of antigen–antibody systems where for some reason the sensitization, cannot be observed by any characteristic reaction.

Any abnormal condition, in which the human red cells strongly adsorbed globulin on their surface, would cause the cells to be agglutinated by the rabbit anti-sera, but as stated above, the RBCs tend to retain their own surface properties even in the presence of large amounts of proteins. The specific reaction between sensitized cells with incomplete antibody and rabbit antihuman-globulin serum seems to be almost conclusive evidence that the “incomplete” antibody is, at least in part, human globulin. Some experiments were recorded back in 1908 on the agglutination of rabbit red blood cells sensitized with a goat anti rabbit-cell immune serum by exposing these washed cells to the serum of a rabbit immunized against goat serum.[3]

At our centre the primary method used for screening all the 26772 healthy donor samples was on IMMUNOCOR Capture R screen platform using SPRCA method.

Tube testing has been the standard of practice until the 1980s. As personnel shortages and cost containment became important issues in the clinical laboratory, automation offered a real solution. Many chemistry, hematology, and immunology laboratories were the first to be automated, but transfusion services were hampered by the complexity of their procedures and the subjectivity of their test interpretation. Because cost containment forced many laboratories to rely on less-experienced and/or rotating staff, solid-phase and CAT methods were especially appealing.[4]

Solid Phase Red Cell Adherence Assay is one of the two tubeless methods that were developed to improve sensitivity and specificity in blood group serology. The SPRCA (solid phase) and the column agglutination (gel) technology have gained wide acceptance following successful adaptation to fully automated platforms, The purpose of this paper is to discuss the development, principle, procedures as well as laboratory and clinical applications of the SPRCA in transfusion medicine.[5]

SOLID PHASE IMMUNE ADHERENCE ASSAY (SPRCA)

Solid Phase Immune Adherence Assay is an immunological technique in which one of the reactants, either the antigen or antibody, is immobilised onto a solid medium and assay for either the antibody or antigen in question. Indicators of end point can be a fluorescein, an enzyme or red cells. When red cells are used as the indicator, the term Solid Phase Red Cell Adherence Assay (SPRCA) is generally used.[5]

Solid Phase Red Cell Adherence Assay (SPRCA) being used today is the culmination of innovations over four decades. In 1956, the technique of mixed agglutination was reported by Coombs and Medford to demonstrate the presence of A and B blood group antigens on human platelets.[6]

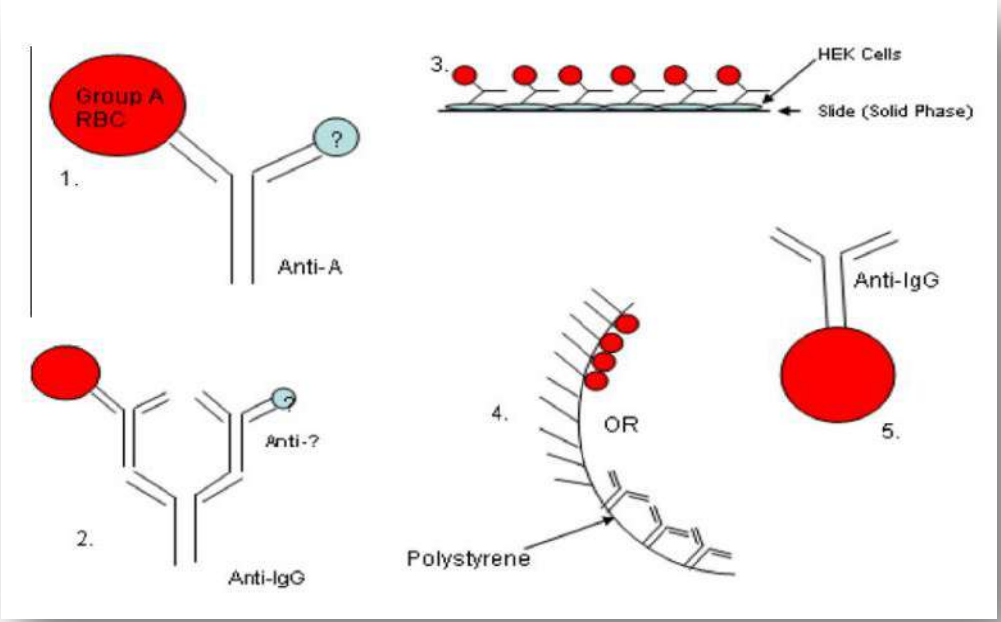


Figure1: Development of SPRCA [5]

The basis of direct mixed agglutination was the linking of a common antigen shared by the indicator cells and the cell in question (Figure 1-1). Indirect mixed agglutination using antiglobulin reagent can also be used to demonstrate other blood group antigens on platelets (Figure 1-2). In 1959, Hogman used human embryonic kidney HEK cells attached onto a slide (solid phase) held horizontally, diluted Anti-A and anti-B were added to the slide and incubated at room temperature for an hour. Slides were washed, group A or B indicator were added and then followed by 1–2 hour incubation. Microscopic examinations of red cell adherence were performed following washing of unbound indicator cells [7] (Figure 1-3). In 1967, Catt and Tregear were able to absorb antibodies or red cells onto plastic polystyrene or polypropylene surface without affecting their immunological activity.[8] (Figure 1-4).

In 1976, Rosenfield et al. reported the use of solid phase assays for pre-transfusion testing, however, the procedure was labour-intensive and data unsatisfactory.[9]

In 1980s, the group from Kansas City, led by Plapp, Sinor and Beck, were able to attach anti-IgG molecules to the indicator red cells immunologically instead of utilizing chromic chloride as outlined by Coombs in the 60s (Figure 1-5). This group and others had shown that

SPRCA can be used to perform routine pre-transfusion type and screen, antibody identification and crossmatch with better sensitivity and specificity than the standard indirect antiglobulin test. In addition, end points could be read manually or automatically by a microplate reader which lends the testing process towards full automation.[10,11]

Commercially available Solid Phase Red Cell Adherence Assays are Capture R Technology™ and Solid Screen II™. Both the methodologies have been licensed and adapted to automation.

An advantage of Solid Phase Red Cell Adherence Assays over the conventional tube method is that the end points are stable and can be read objectively by technologists or by an optical sensor of an automated instrument.[3]

Immucor's Capture R Technology

Immucor Inc. first marketed the Capture technology for red cell and platelet serology in the mid 1980s.[3] A plastic microtitre plate of 96 U-bottomed wells (8 12) was used as solid phase. In the antibody detection test, a chemical bonding agent was used to adhere reagent red cells to the wells. In the mid 1990s, the company began marketing the second generation of the Capture R Ready Screen™ technology using red cell membranes instead of the convention viable red cells. In addition, individual 12, 8-well strips were used to replace the classical 12, 8 miro-titre plate. The new design offers several advantages:

1. Red cell membranes can be stored at room temperature and have longer shelf life of 12 weeks.
2. Pre-coated red cell membranes reduce one step less to the test procedure.
3. Increased serum to ''cell'' ratio thus increasing sensitivity as red cell membrane occupies little volume in the test mixture.
4. Testing strips can be used to reduce wastage.

Principle of Capture R Ready Screen [12]

Reagent screening cell membranes are immobilised by a proprietary bonding agent to the bottom of the test wells.

Unexpected red cell antibodies from plasma sample are sensitised to specific antigens during incubation in LISS.

Unbound red cell antibody and other plasma proteins are washed off. One drop of a 0.5% indicator cells coated with murine monoclonal anti-IgG is added to each well and the strip is then centrifuged at 530xg for 2 min.

Procedure

1. Add two drops of LISS additive followed by one drop of plasma into each one of the three wells containing red cell membranes of Group OR1R1, R2R2 and rr. The fourth well contains pre-coated IgG used as a procedural positive control. Therefore, two samples can be tested on each strip.
2. Incubate the plate at 37^{o}C for 15 min in a water bath or 20 min in a dry heat incubator.
3. Wash 2 times by a semi automatic micro titre plate washer or 6 times manually.
4. Add one drop of the Capture Indicator Cells to each well.
5. Centrifuge plate at 530xg for 2 min.
6. Place the wells onto an illuminated surface and record effacement (positive reactions) or red cells buttons (negative reactions).

Reading Results

1. Positive reactions

The solid phase bound IgG's ‘‘Capture’’ the anti-IgG attached indicator cell from being spun down thus forming a confluent layer of red cells (effacement of indicator red cells). That is, no indicator cell button at the bottom of the well as they have been captured by the solid phase bound IgG.

2. Negative reactions

If the sample does not contain red cell specific antibody, there will not be any solid phase bound IgG present to capture the indicator cells. As a result, the indicator cells are ‘‘spun down’’ to form a cell button.

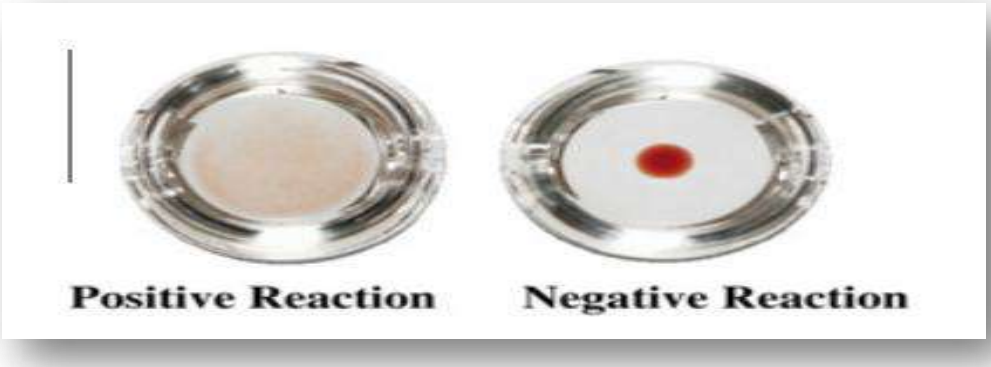


Figure 2: Reading of Results in Capture R screen [5]

Figure 3: Grading of Results in Capture R screen [5]

Applications of SPRCA [3]

1. Direct antiglobulin test

Capture R: A monolayer of patient's red cells are adhered to the bottom of the well by a bonding agent in the Capture Select strip, excess red cells and free IgG are washed off before one drop of IgG coated indicator cells is added, and the strip is spun and read. For Capture R, only IgG is detected.

2. Weak D testing

Capture R: A monolayer of patient's red cells are adhered to the bottom of the well by a bonding agent in the Capture Select strip, reagent anti-D is added and incubated at 37 C for 20 min (15 if water bath or Echo is used), wells are washed to remove IgG before one drop of IgG coated indicator cells is added, and the strip is spun and read.

3. IAT crossmatch Similar to weak D testing having reagent anti-D replaced by patient's plasma.

4. IAT phenotyping Similar to weak D testing except using specific reagent antiserum.

5. SPRCA applications in Platelet Serology

6. Others: SPRCA for detecting antibody to cytomegalovirus and syphilis have been developed commercially and it has potential applications for high volume screening for other viral or bacterial infectious markers.[13]

Timely diagnosis of immunological causes of Transfusion Related Acute Lung Injury (TRALI) using SPRCA can be potentially developed. Finally, in blood group serology, weaker than normal expression blood group antigens may be better detected by SPRCA. Weak subgroups of A and B, weak expressions of C, E, c and e in rare Rh haplotypes, Fyx, weak expression of Kell system antigens in Mcleod phenotypes are just a few of many examples.[3]

COLUMN AGGLUTINATION TECHNOLOGY

In the year 1988, Yves Lapierre, working with DiaMed AG in Switzerland, reported using column agglutination technology (CAT) to perform RBC typing and antibody screening tests.[14] Dr. Yves Lapierre of Lyon, France, investigated gelatin, acryl amide gel, and glass beads as he searched for a way to trap red blood cell (RBC) agglutinates during standardized sedimentation and centrifugation. The ideal material for trapping the agglutinates is provided by the gel particles, and this discovery led to a patented process that is the basis of the gel-based tests.

Studies were conducted to see if this CAT Antiglobulin test gave satisfactory results. **Reis et al** worked on serum and plasmas of 228 samples for red cell antibodies with two anti-human globulin reagents: one containing only anti-IgG and the other containing both anti-IgG and anti-C3b, -C3d. After the initial testing, there was 94% agreement between column agglutination technology and tube tests, and after repeat testing, there was 97% agreement.[15]

At the same time, it was also discovered that antiglobulin tests can be performed without multiple saline washes to remove unbound immunoglobulin. Red blood cells and plasma are allowed to interact in

air bubble barrier of chamber to keep unbound plasma from neutralizing the antiglobulin reagent that is located in the lower gel column. Because unbound plasma and antiglobulin reagent are prevented from mixing, antiglobulin control cells are not needed to confirm the presence of antiglobulin reagent in negative tests.

Compared with traditional tube technology, the gel-based tests provide a more stable endpoint and more reproducible results—eliminating the variability associated with the physical re-suspension of RBC buttons after centrifugation and the subjectivity in interpretation of hemagglutination reactions.

A BIT MORE HISTORY

Ortho Clinical Diagnostics, Florida in the year 1994 began distributing the ID-MTS Gel through Micro Typing Systems (MTS), Inc. In 2002, Ortho Clinical Diagnostics, Inc. acquired MTS, and its gel-based test has been named the ID-Micro Typing System (ID-MTS).[16] In July 2013, the FDA granted Grifols Diagnostics Solutions, Inc.[17] a license to manufacture and distribute DG Gel 8 cards, [17] a form of CAT, in the United States. The major one difference between these two products is the number of columns. The ID-MTS contains 6 columns, while the DG 8 Gel contains eight columns. Both companies offer automated equipment for their products.

Principle of Gel test

The gel test, which is performed in a specially designed microtube, is a hemagglutination test based on controlled centrifugation of RBCs through a dextran-acrylamide gel that contains predispensed reagents. Each microtube is composed of an upper reaction chamber that is wider than the tube itself and a long, narrow portion referred to as the column. A plastic card with microtubes is used instead of test tubes in the gel test. A gel card measures 5 × 7 cm approximately and consists of 6 or 8 columns. Each microtube contains predispensed gel, diluent, and reagents, if applicable. Measured volumes of RBCs and serum or plasma are dispensed into the reaction chamber of the microtube. If appropriate, the card is incubated and then centrifuged. The reaction chamber is where RBCs may be sensitized (antigen-antibody binding)

during incubation. The column of each microtube contains dextran-acrylamide gel particles suspended in a diluent with reagents added, if applicable.

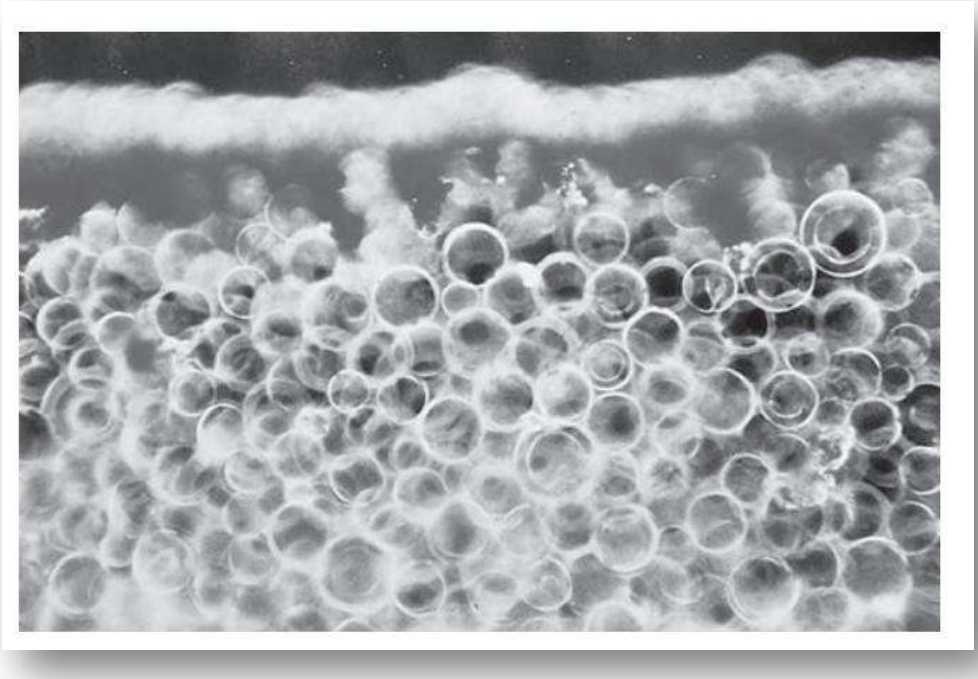


Figure 4: Ortho Gel Matrix (close up) [4]

The gel particles make up 75% of the gel-liquid mixture that is preloaded by the manufacturer into each microtube.

In structure the gel particles form a porous location, and they serve as a reaction medium and filter, sieving the RBC agglutinates according to size during centrifugation. Large sized agglutinates are trapped at the top of the gel and are not allowed to travel through the gel during the centrifugation of card. Agglutinated RBCs stay trapped in the gel, while unagglutinated RBCs travel unimpeded through the length of the microtube, forming a pellet at the bottom following centrifugation.

The column agglutination technology AHG test eliminates the need to wash red cells, which decreases the overall test time. It becomes easy to perform the test, and the results are more objective than those with tube and microplate methods.[15]

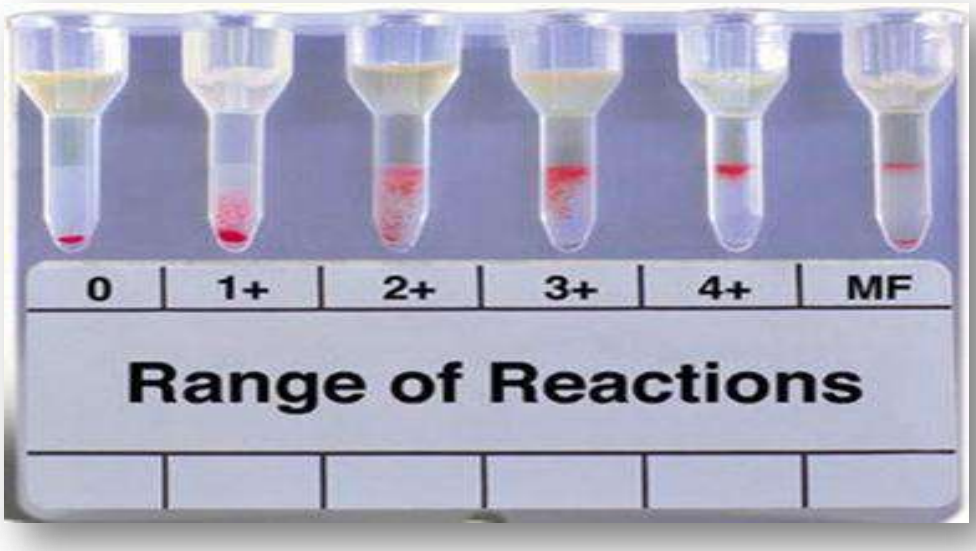


Figure 5: Range of reactions in Indirect agglutination test on gel card.[16]

Localized Red blood cell patterns resulting from the indirect agglutination test in ID-Micro Typing System™ gel cards (Ortho Clinical Diagnostics, USA). Agglutination scores below the tubes indicate agglutination strength, from 0 (no agglutination) to 4+ (strongest agglutination). The rightmost column shows an example of a mixed readout (MF) [18]

Results and Grading

Agglutination reactions in the gel test are graded from 1+ to 4+ and mixed-field, similar to the reactions in the test tube hemagglutination technique.

4+: solid band of agglutinated RBCs at the top of the column. Usually, no RBCs are visible at the bottom of the column.

3+: most of the agglutinated RBCs remain near the top of the column, with a few agglutinates staggered below the thicker band and majority of agglutinates are seen in the top half of the column.

2+: RBC agglutinates are distributed throughout the upper and lower halves of the column, with a few agglutinates at the bottom of the microtube.

1+: RBC agglutinates are predominantly seen in the lower half of the column, with some RBCs at the bottom of the microtube.

Weak Reaction: with only a few agglutinates remaining in the area just above the RBC pellet at the bottom of the microtube.

Negative reaction: RBCs form a well-delineated pellet at the bottom of the microtube.

In a **mixed-field**(mf), the reaction is characterized by a layer of agglutinated RBCs at the top of the column and a pellet of unagglutinated cells at the bottom of the microtube.

False-positive mf reactions may be caused when incompletely clotted serum is used in the CAT test. Fibrin strands in such serum may trap unagglutinated RBCs, forming a thin line at the top of the column. Other unagglutinated cells pass through the column during centrifugation and travel to the bottom of the microtube. The clinical history of the patient should be considered before interpreting a reaction as a mixed-field. For example, recently transfused patients or bone marrow transplant recipients are expected to have mixed populations of RBCs, and their RBCs commonly produce mixed-field reactions. Hemolysis is seen as clear red liquid in the chamber.

CAT tests may be read manually or using interpreter in the form of Readers provided by various platforms.

After the initial detection of antibody in the sample, it is the turn to screen it broadly, then pinpoint and identify the type of antibody.

ANTIBODY IDENTIFICATION

Goal of antibody identification

It is to determine what caused a positive antibody screening and/or crossmatch, or positive direct antiglobulin test (DAT). Once the antibody(ies) is(are) identified, then transfusion services can select the appropriate blood component or therapy for patients and resolve cases suspected Hemolytic Disease of the Fetus and Newborn (HDFN). Antibodies identified in the plasma/serum of blood donors can be useful for testing the red cells of donors, research and/or teaching purposes, as the plasma will not be used for transfusion.[19]

All laboratories try their best to provide the best antibody identification; hopefully, using a basic approach to resolve the problems with the

least amount of steps.[19] Approaches though are varied, but several steps to consider are:

(1) What is required,

The requirement is to have methods of testing to demonstrate clinically significant antibodies i.e. means of incubating at 37^{o}C before performing an antiglobulin test (by reading tests at antiglobulin phase).

(2) Test methods,

Manual tube tests, microplate, gel, solid phase and automated techniques are various methodologies used for antibody identification that almost always use low ionic strength salt (LISS) solutions. The FDA requires that all group O reagent screening cells and panel cells be typed for these antigens: (D, C, E, c, e, from Rh blood group system), (K, k, from Kell Blood group system), (Fy^{a}, Fy^{b}, Duffy Blood Group System), (Jk^{a}, Jk^{b}, Kidd Blood Group system), (Le^{a}, Le^{b}, from Lewis Blood Group), P1, (M, N, S and s from MNS Blood Group System).[20]

(3) Thermal range (pro and con),

There are only few that will incubate and read their antibody identification tests in multiple phases; i.e. room temperature, 37^{o}C and antiglobulin tests. The pros for identifying antibodies that react in all phases is that all reactions will be explained, especially when there is an extraneous reaction or two at antiglobulin phase for which a specificity cannot be determined. The con to identifying antibodies that react before the antiglobulin test is that these antibodies may not have any clinical significance and require additional unnecessary testing.[19]

(4) Limited thermal range agglutinins,

It must be kept in mind, that certain antibodies usually react only at a particular phase of testing. Lewis, P1 and MN antibodies generally are known for reactivity at room temperature. Ss, Duffy and Kidd antibodies are reactive best in antiglobulin phase.[19]

(5) Broad thermal range agglutinins,

These antibodies very often react in more than one phase of testing. They include Rh and Kell system antibodies.[19]

(6) Single specificity,

When an antibody is identified, there are at least three cells on a panel positive for an antigen, K for instance, that are reactive and three cells negative for K that are nonreactive. If all K negative cells are positive for all the other represented common antigens and are non-reactive, then there are no other antibodies present.[19]

There are some laboratories that require only two reactive and two nonreactive tests for positive identification – AABB Immunohematology Reference Laboratories (IRL) [21]

(7) Multiple specificities,

When more than one antibody is present, a pattern for one or more of the antibodies could immediately be obvious. E.g; all K and S positive cells are reactive and K negative S negative cells are all non-reactive. This time, K positive reactive cells would have to be S negative, and the S positive cells should be K negative to determine if either specificity was present, using the three reactive, three non-reactive rule. It is not necessary to test another complete panel of cells when the original panel does not have the required number of cells. Rather, selected cells can be picked from other panels to confirm the antibodies.[19]

(8) Alloantibody vs autoantibody,

Autologous control is the most important cell for testing in antibody identification. With time several laboratories have dropped the autologous control with the antibody screening, but it is essential in antibody identification. If the ‘‘auto” control is non-reactive, then the antibody is most probably alloantibody. If control is positive, then it should be determined if it is mixed field, indicating that the patient has been recently transfused, check the history. If the autologous control is positive, but does not appear to be mixed field, then the antibody(ies) in the sample could be autoantibody, showing a blood group specificity such as anti-K.[19]

(9) Negative screening with a Positive crossmatch,

A positive crossmatch can occur with a negative antibody screening when there is an antibody present that is directed at a low incidence antigen not on the screening cells. If the antiglobulin crossmatch is

performed, then the antibody will be detected. If it is not performed, the antibody may not be recognized unless there is a transfusion reaction reported.[19]

(10) Negative Antibody screen with Positive DAT

This is not a common occurrence, is not obvious in routine situation, as DAT should be mentioned in routine testing. Possibility of a very weak beginning alloantibody could be coating recently transfused donor red cells, causing a positive DAT, but not yet demonstrated in the plasma/serum of the patient.[19]

(11) Hemolytic Disease of the Fetus and Newborn (HDFN) investigation, In most cases of HDFN, the mother's plasma/serum contains an alloantibody directed at an antigen on the baby's red cells that is inherited from the father. The mother's antibody is identified and when the baby is born, the red cells may or may not have a positive DAT. [19]

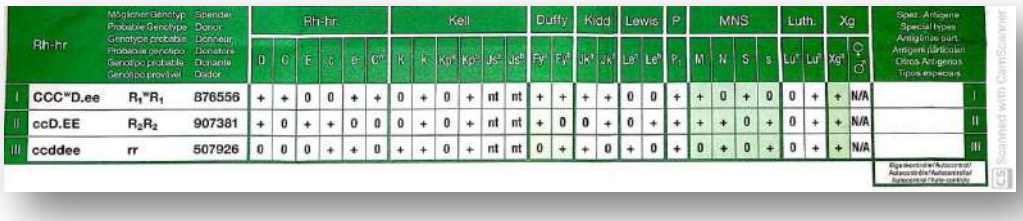
(12) Special techniques.

When the antibody identification process does not yield clear cut results, special techniques can be used to assist in enhancing an antibody, or the opposite, eliminate the reactivity of the antibody by treating the test cells to destroy the antigen. Use of Enhancement Media like PEG, enzymes like ficin and papain and chemical treatments like DTT can be done.

Ficin and papain also destroy certain antigens, MNSs and Duffy, allowing them to be easily confirmed in antibody mixtures with other antibodies that react or are enhanced. Certain chemicals, DTT in particular, also can aid in identifying and/or confirming certain antibody specificities. The Kell system antigens are destroyed by treatment with DTT, as are others.[19]

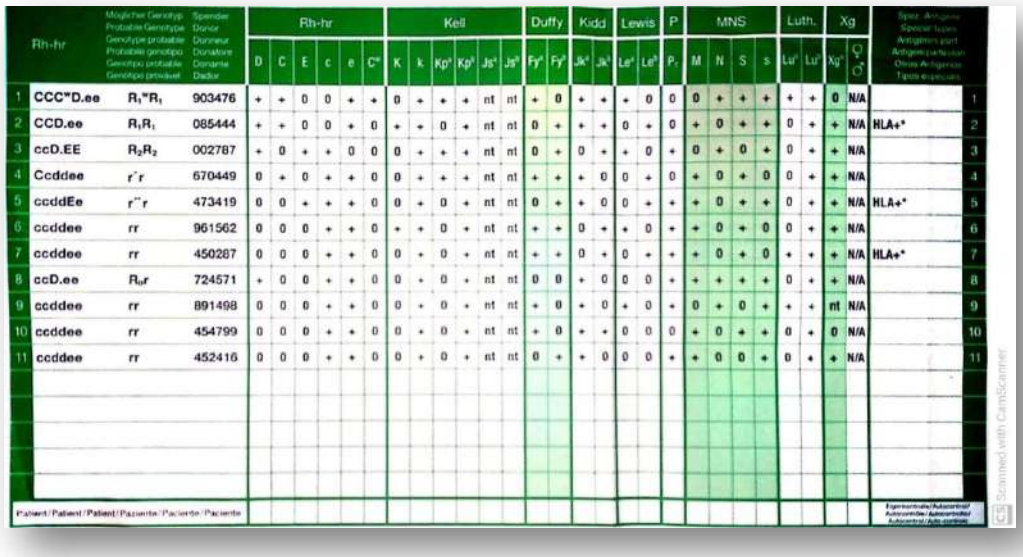
Inhibition/Neutralization Techniques are employed to assist in confirming antibody specificity, rule out other alloantibodies determine whether an antibody is IgM and/or IgG. Various fluids or chemicals are added to serum or plasma, with a dilution control tested in parallel, to abolish antibody activity.

During our Study we used Bio-Rad 3 cell and 11 cell Panel in our course to identify. Following figure 6 and figure 7 shows Antigrams of one such commercial panel.



	Rh-hr			Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg	
				D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^a	♀ ♂
I	CCC^{w}D.ee	$R_1{}^wR_1$	876556	+	+	0	0	+	+	0	+	0	+	nt	nt	+	+	+	+	0	0	+	+	0	+	0	0	+	+	N/A	I
II	ccD.EE	R_2R_2	907381	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	+	0	+	0	+	+	N/A	II
III	ccddee	rr	507926	0	0	0	+	+	0	+	+	0	+	nt	nt	0	+	+	0	+	0	+	0	+	0	+	0	+	+	N/A	III

Figure 6: Bio-Rad 3 cell Panel Anti-gram for screening [22]



	Rh-hr			Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg	
				D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^a	♀ ♂
1	CCC^{w}D.ee	$R_1{}^wR_1$	903476	+	+	0	0	+	+	0	+	+	+	nt	nt	+	0	+	+	+	0	0	0	+	+	+	+	+	0	N/A	1
2	CCD.ee	R_1R_1	085444	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	+	0	+	0	+	0	+	+	0	+	+	N/A	HLA+*	2
3	ccD.EE	R_2R_2	002787	+	0	+	+	0	0	0	+	+	+	nt	nt	0	+	0	+	+	0	+	0	+	0	+	0	+	+	N/A		3
4	Ccddee	r'r	670449	0	+	0	+	+	0	0	+	+	+	nt	nt	+	+	+	0	0	+	0	+	0	+	0	0	+	+	N/A		4
5	ccddEe	r''r	473419	0	0	+	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	+	+	+	0	+	+	0	+	+	N/A	HLA+*	5
6	ccddee	rr	961562	0	0	0	+	+	0	+	+	0	+	nt	nt	+	+	0	+	+	0	+	+	0	+	0	0	+	+	N/A		6
7	ccddee	rr	450287	0	0	0	+	+	0	0	+	0	+	nt	nt	+	+	0	+	0	+	+	+	0	+	0	+	+	+	N/A	HLA+*	7
8	ccD.ee	$R_{o}r$	724571	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	0	0	0	+	+	+	+	+	0	+	+	N/A		8
9	ccddee	rr	891498	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	+	0	+	0	+	0	+	+	+	nt	N/A		9
10	ccddee	rr	454799	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	+	0	0	0	+	0	+	+	0	+	0	N/A		10
11	ccddee	rr	452416	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	0	+	+	0	0	+	0	+	+	N/A		11

Figure 7: Bio-Rad 11 cell Panel Anti-gram for Identification [23]

Solving the antigrams of each panel takes a lot of practice and experience.

Every Instituite has their own set of rules for solving them and inference for results. We followed steps mentioned in the book Modern Blood Banking and Transfusion Practices Harmening 7th ed.[4]

ALLOIMMUNIZATION TO RBC ANTIGENS

The process of development of non-ABO antibodies following RBC transfusion, pregnancy, or transplantation is known as alloimmunization.

How does it affect us in blood transfusion services?

1. It is a substantial challenge in completing compatibility testing in a timely manner or even obtaining antigen-negative blood.

2. It complicates patient care and increases the risk of acute and delayed hemolytic transfusion reactions.

GENERAL INCIDENCE OF ALLOIMMUNIZATION IN COMMUNITY

A varied incidence of alloimmunization occurs according to clinical setting studied and condition of the patient.[4]

The incidence of alloimmunization among healthy Blood Donors is less than 0.3%, unselected hospitalized patients who have had **type and screen** testing ranges from about 1% to 3% and in surgery patients post-transfusion alloimmunization is around 3%.[4]

TYPE AND SCREEN: After testing a patient's blood for ABO, Rh, and unexpected antibodies (antibody screen), if no abnormalities exist in the ABO and Rh and no unexpected antibodies are detected in the antibody screen, then the recipient blood sample is retained in the event that subsequent serologic crossmatching is necessary.[4]

In chronically transfused patients like sickle cell disease, myelodysplastic syndrome, thalassemia, or autoimmune hemolytic disease the prevalence of alloimmunization is much higher, 30% or greater.[24]

SCIENCE OF ALLOANTIBODY FORMATION and ITS CLINICAL SIGNIFICANCE

1. Antigen inducing immunological response: D antigen has a strong response to RBC protein antigens. About 85% of healthy D-negative persons exposed to the D antigen will develop anti-D. Other antigens causes much lower sensitization: anti-K develops in about 10% of K-negative individuals exposed to K antigen, anti-E in about 7%, and anti-c in about 3%. Thereby minor blood group antigens produce alloimmunization in less than 3% of antigen-negative persons in the study conducted by **Körmöczi et al**.[24]

2. Underlying pathology: Diseases like AIHA, SCD, cause of anemia, cumulative number of transfusions, and the **immunogenicity** of the non-self RBC antigens to which the patient is exposed.[25] It will be explained more detail with various studies conducted.

3. Individuals Susceptibility: These persons, called responders, represent the majority of patients identified with alloantibodies. They have been categorized as

- **Responders**
- **Hyper responders**: Among alloimmunised patients/individuals, studies have shown about a quarter of them develop more than one alloantibody.
- **Non Responders**

4. Clinical and biological factors associated with responders: These may include genetics, the inflammatory state of the patient, sex, and age.[24,25] Patients with underlying systemic inflammatory disorders have been are more likely to develop alloimmunization.

5. Antigen diversity between donor and recipient populations may explain some alloimmunization, particularly in patients with sickle cell disease. Individual difference in the HLA class II system with respect to antigen presentation is another genetic variable that might explain responsiveness.

6. Studies regarding the gender of the patient with regard to alloimmunization are conflicting, and there is no evidence that clearly identifies a predisposition toward or protection from alloimmunization for either sex.[24]

7. Neonates, young children, and the elderly appear to be **poor responders**.[24]

The rate of alloimmunization in patients with sickle cell disease (SCD) is much higher than the general population, ranging from about 19% to almost 50% in different studies. Patients with a higher cumulative number of transfusions have a higher rate of alloimmunization.[25]

Furthermore, a high prevalence of alloimmunization is seen even with policies to provide blood with extended phenotype crossmatching and from African American donors.[26]

Anti RBC antibodies detected in the field of transfusion practice are produced by humoral response to the alloantigens during previous

encountered foreign antigen exposure. Sheep red blood cells have been used as a model antigen for years.[27]

RBCs transfused through blood appear to be only weakly immunogenic. The current picture is still not clear whether RBC antigen response is qualitatively different from general mechanisms of immune response to foreign antigen.

Differences between traditional microbial or protein immunogens and transfusion of sterile blood have been noted in relation to Route of immunization, potential absence of inflammation and danger signals, dose and kinetics of antigenic exposure, type of antigen-presenting cell involved, and the molecular nature of the antigen. Exact role of these variables is still under study but they have been explained theoretically.

The two main factors affecting frequency of Alloimmunization to RBC Transfusion are Immunity and Tolerance. Studies have shown even under numerous foreign epitopes on all transfusions of non-autologous RBCs, transfusion is not a highly immunogenic. Response to multiple transfusions, alloimmunization to alloantigens on transfused RBCs has an frequency of approximately 2% to 6%.[28-30]

One study revealed alloimmunization rates in relation to underlying disease: lymphocytic leukemia 0%, gastrointestinal bleed 11%, aplastic anemia 11%, renal failure 14%, myelogenous leukemia 16%, and hemoglobinopathy 29%.[31] A trend is shown with a clear decrease in lymphocytic leukemia, presumably due to immunosuppression. Previously, it has been proposed that alloimmunization is unusually high in some disease states, such as sickle cell anemia, myelodysplastic anemia, and autoimmune hemolytic anemia.

Some studies reported that leucoreduction may decrease immunogenicity of transfused RBCs.[32] Since use of leucoreduced blood varied in the many already conducted studies, this may be a relevant issue.

One more complicating factor is most of the studies are retrospective and do not compare pre-transfusion specimens to post-transfusion specimens so it is unclear if the presence of an alloantibody is necessarily the result of an antecedent transfusion.

Formation of Autoantibody and Science behind it is

Loss of immunologic tolerance to self-tissues can result in autoimmune processes targeted against specific organs or self-tissues. Autoimmune pathologies known to us, it is seen in generation of autoantibodies against self-RBC antigens. This results in a clinically-silent auto-agglutinin that is detected incidentally by a positive direct antiglobulin test (DAT) during alloantibody screening e.g AIHA.[27]

Specificities of the most common of alloantibodies and their associations were determined to correct management of RBC transfused. A retrospective study was done between 2013 and 2015 in laboratories at the Blood Transfusion Center of Rabat in **Morocco**. Variables like: frequency, specificities of alloantibodies, blood group involved in alloimmunization and difficult of management of transfusion in case with association of alloantibodies were studied.[32]

500 of alloantibodies were identified in 425 people out of which 53 were blood donors and 372 were patients/pregnant women. Alloantibodies were formed against: RH1 (50.8%), RH3 (11.4%), KEL 1 (8.2%), RH2 (7.6%), RH4 (4.6%), MNS1 (4%), MNS3 (2.6 %), Jk^a (2.4%) and Fy^a (2.2%). Only one alloantibody was seen in 85% and in remaining 15% at least two alloantibodies were found.[33]

They concluded that Red blood cell immunization is a serious problem in transfused patients. Those associated with low frequency antibodies should be encouraged to donate.[34]

A survey of 6 years(1969-1975) has demonstrated that an average of 2.2% per year of individuals requiring compatibility testing had antibodies de novo and frequency for individuals forming antibodies following transfusion is 4.1%.[34]

Detection rate increased from 1.5% (1969) to 4.1% (1971–1975) was almost entirely due to the sensitivity of the two-stage papain technique. A total of 124 antibodies were detected, out of which 3 enzyme-active antibodies were found to be the cause of mild hemolytic transfusion reactions. 60% of the listed 332 alloantibodies were Rh-specific and hence the transfusion of appropriately Rh phenotyped blood is strongly recommended.[34]

To assess the true magnitude of the problem is paramount to make such necessary changes in transfusion policies.[35] Performing regular antibody screening and meticulous reporting of RCA after platelet transfusion makes the first step toward safe approach.

Red cells in platelet concentrates can induce de-novo antibody formation which is primary immunization or act as a booster to an unknown stimulus leading to secondary immunization.

It would be ideal to do screening tests on two different occasions following transfusions:-

i) around 3–7 days to detect early appearing antibodies or booster effect and

ii) around 4–8 weeks to detect slowly developing antibodies.[35]

After many years Pre-transfusion testing has evolved for the better and taken up a rational approach. This has resulted in a more efficient transfusion service but with no compromise to patient safety. A debate is undergoing currently within various centres on the necessity of performing an antiglobulin crossmatch if a suitable screening test for detecting red cell antibodies is negative.

A retrospective study by **Peterson et al** was taken of all pre-transfusion testing in order to determine the specificities and possible clinical significance of antibodies detected by the crossmatch alone. The results favored omission of the crossmatch will not compromise patient safety.[36]

Its beneficial for the patient as less time is needed to provide homologous donor blood in this method. Its cost effective for the laboratories as a result of less antiglobulin reagent being used, less time taken for providing and less blood needing to be held in stock.

Thus better part of monetary funds can be channel into areas that will result in greater patient safety at a lower cost than cross-matching.[36]

Two of these areas are prevention of patient and specimen identification errors by the use of a unique transfusion number system and the full utilization of an autologous blood service.[36]

INCIDENCE of Unexpected Antibodies among DONORS

If a large amount of plasma or whole blood is transfused as in the cases of massive transfusions or in pediatric population in presence of clinically significant unexpected antibodies can occasionally cause severe transfusion reactions. In such cases it is advisable to preferably transfuse PRBC.

4 year survey of the incidence of iso-immune antibodies in a volunteer donor population was reported by **Myhre et al**. A yearly average of 0.34% of all the units of blood was found to contain iso-immune antibodies. Antibodies were found in 0.56% of first time donors.[37] Blood donors should be tested for infectious markers for blood safety along with for irregular antibodies should be performed for safe and compatible blood transfusion, especially for previously alloimmunized individuals.[38]

RBC alloantibodies, especially IgG immunoglobulin class, are clinically significant because of the association with hemolytic disease of newborns (HDN), hemolytic transfusion reaction (HTR) and a significant reduction in lifespan of transfused red blood cells. [39] **Babaei et al**. cross sectional study conducted in Iran on 75 donors of Zanjaian population by alloantibodies were detected in serum of 6 donors i.e alloimmunization rate came to be 8%. This study had quite a varied results ranging 0.5%-60% depending upon different selected population and method sensitivity. Among 75 donors 73 (97.33%) males and 2 (2.66%) females were there. In this study frequency of blood groups were A (52%), O (29.33%), B (14.66%) and AB (4%), Rh+(97.33%) and Rh-(2.67%). The most prevalent alloantibody was against K (66.66%), c (16.66%) and e (16.66%) antigens.[39]

The selection of suitable products for the transfusion becomes easier with Identification of donors' antibodies, so the risk of complications of blood transfusions are greatly reduced as was shown by **Eder et al**.[40]

Nicholls MD has shared the concern about lack of enough literature on the prevalence of RBC alloantibody in the general population. Reported incidence of RBC alloantibodies in these literatures varied significantly.[41]

Tormey et al. studied military veterans which showed a high incidence for obvious reason of prior blood transfusion. Hence results are affected by selected population and laboratory test sensitivity.[42]

El-Faramawy et al.[43] carried out study on 200 healthy Egyptian blood donors coming to Ain Shams University Hospitals Blood Banks. Samples from donors were screened via IAT method. In this study 130 (65%) were male donors and 70 were female donors. Alloimmunisation rate was 4% i.e in 8 donors, 5 (62.5%) females and 3 (37.5%) males. One female had history of previous transfusion. 3 were multipara and 2 were single. Alloantibodies found were Anti-Kell (k)(12.5%), anti-C (12.5%), anti-M (12.5%), anti-c (12.5%), and anti P (12.5%), and 37.5% of positive cases were non-specific antibodies. Frequency of blood groups in their population were A (35.5%), O (32.5%), B (22%), AB (10%), and as regard Rh (97%) had Rh positive and (3%) were Rh negative.[43]

Giblett et al.[44] had reported low incidence of 0.32%.RBC alloantibodies in blood donors.

Winters et al.[45] reported 0.89% prevalence among blood donors of Olmsted county, Minnesota that consisted of donors who were previously transfused and pregnant women in their study, which probably explains the higher percentage of alloantibodies.

Shafini et al.[46] study showed 0.8% (four out of 500) prevalence of RBC alloantibody among the blood donors in a tertiary hospital in the east coast of Malaysia.

Ameen et al.[47] had conducted study on 179,045 Kuwaiti pregnant women, and allogeneic blood donors and patients the prevalence of alloantibodies was 0.49 %.

Lower alloantibodies incidence was detected in a study done by **Pahuja et al.**[48] which was 0.05%, in their donor population counted 7756, 7648 donors were males (98.6%) and 108 were females (1.4%), this very low incidence of alloantibodies may be due to the very low percentage of females in the study, who are known to be of higher probability for alloantibody formation.

Garg et al.[49] among 47,450 whole blood donors reported incidence of 0.09% for red cell alloantibody same as found by **Makroo et al.**[38] in his study in India among 82,153 donors, 93.40% were males and only 6.60% were females.

Other studies showed higher incidence in southwestern Iran by **Keikhaei et al.**[50] in which the prevalence of alloantibodies was 18.7%, a near result to 17.9% in southeastern Iran by **Mirzaeian et al.**[51]

Other Egyptian study by **Ahmed et al.**[52] including 501 subjects was done and revealed an alloimmunization rate of 11.3%, these high incidences are explained by the different type of population in these studies, as the subjects were thalassemic patients in contrast to the healthy donors.

Reyhaneh et al.[53] conducted one study in Ardabil in which the most prevalent of alloantibody was against K (30%), E (15%) and c (15%) antigens, similar results seen in Tehran by same group i.e Anti-Kell (23.53%), Anti-E (20.59%), and Anti-c (17.56%).

Vaziri et al.[54] in Yazd (Iran) in patients with beta thalassemia, the rate of prevalence of alloantibody was 4% that these antibodies were against Kell, C and D antigens. Another one from northwest of Iran by **Davari et al.**[55] in patient with beta thalassemia major, the rate of prevalence of alloantibody was 16.32% that these antibodies were against Kell, E and c antigens.

Makroo et al.[38] study showed Anti-M was the most common antibody identified (56.57%) followed by anti-D (27.63%), then the frequency of anti-E and anti-C was found to be (2.63%) each which is lower than our and other studies mentioned above.

Other study done by **Kaur et al.**[56] also detect Anti-M as the most common antibody identified (42.8%), anti-le^a (14.2) and 14.2% were inconclusive.

In **El-Faramawy et al.**[43] three(37.5%) positive cases were found to be non-specific, a result going with **Kaur et al.**[56] study at which 1 case (14.2 %) was inconclusive.

One admirable thing to be noted in all the studies conducted is automation has improved the objectivity and reproducibility of tests. It

reduces human errors in patient identification and transcription errors. Another plus is documentation and traceability of tests, reagents and processes and archiving of results.[57] Hence in most of the conducted studies we can conduct several testing on small amount of sample and more satisfactory inferences can be derived.

Cumulative incidence of irregular antibodies amongst patients (n=32,560) and donors (n=31,367) were 0.12 and 0.009%, respectively. Commonest antibodies were from Rh system with anti-D being the most common antibody type (14/40; 35%) while in donors it was the MNS system with anti M (2/3) being the most common in the study done by **Tiwari et al**.[58]

Screening of 15,033 random donor antibodies in Shao-guan area under **Zhu et al** [59] by screening cells, poly-brene and anti-globulin tests and in results irregular antibodies were found in 42 samples. The frequency of irregular antibodies in female was found to be higher in male populace and Rh blood group antibodies such as anti-D, anti-E, and anti-C were common (47.6%).

Hamilton et al studies on the frequency of red cell alloantibody formation have shown that approximately 2-3% of patients exposed to red cell antigens will form alloantibodies.[60]

Garcia et al.[61] observed Female dominance among donors was observed in their study similar to other two studies above.

Overall prevalence of alloantibodies in both patients and donors when using conventional tube techniques was 0.7%; for patients only was 0.9% and donors 0.6% in **Promwong et al** [62] Frequent antibodies detected in both groups were anti-Le(a), anti-Mi^{a}, anti-Le(b), anti-P1 and anti-E. Alloantibodies detected via CAT were found in 0.8% of patients and 0.13% of donors with the five most common alloantibodies being anti-Mi^{a}, anti-E, anti-Le(a), anti-c and anti-Le(b). Similarly, common alloantibody specificities in donors were anti-Le(a), anti-Mi^{a}, anti-Le(b), anti-M and anti-D in the Promwong study.

Varying of results i.e. non specific could be False positive may be due to the different techniques used for antibody screening and identification. In **Makroo et al**. [38], antibody screening and identification

was performed by Solid Phase Red Cell Adherence Assay (SPRCA) technique, whereas, other studies used column agglutination technique. The sensitivity of antibody detection by (SPRCA) is higher as compared to column agglutination.[54]

Patidar et al [63] study conducted within period October 2017 to July 2018 with total 18112 donors. Out of them a total of 17 (0.09%) alloantibodies and 1 (0.01%) auto antibody. Out of 17 alloantibodies, 7 were anti-M (3 warm reacting and 4 cold reacting), 3 anti-E, 3 anti-Le^a and one each of anti- K, anti-N & Le^a (combined), anti-C^w and one unidentified alloantibody detected. We also detected one autoantibody.

Erikstein et al [64] study showed the detection and immunization frequencies for the whole cohort were 0.39% and 0.51%, respectively, whereas the RBC alloantibody prevalence was 0.73%. The most frequent RBC alloantibodies were anti-E (20.1%), anti-M (18.7%), anti-K (9.8%), anti-D (8.9%) and anti-Fy(a) (7.0%). In pregnant women, the most frequent were anti-M, anti-D and anti-Le(a) (20.8%, 18.9% and 18.9%, respectively), whereas there was no anti-K detected. Anti-E and anti-M were the dominating RBC alloantibodies in the pre-transfusion testing of in-hospital patients (24.1 and 17.1%, respectively). Eighteen (9.2%) persons in the total cohort had two RBC alloantibodies, six persons had three alloantibodies, and two persons had four alloantibodies. Rh and K typing to prevent future immunisations was only performed in 21.0% of the individuals who presented with a new alloantibody; despite that, 50.5% of the detected alloantibodies had such specificities.

The most commonly encountered immune antibodies are D, K, E, Fy^a and Jk^a.[65,66]

In **Thakur et al** total 4573 blood donors, 88.2% were males. Most of the donors (77.6%) were in 18-25 years of age group. The commonest ABO blood group present was B (36.1%) followed by O (29.1%), A (24.8%) and AB (10%) in blood donors; while in Rhesus system, 95.0% donors were Rh-positive and 5.0% donors were Rh negative. Quite contrary to all the studies discussed above, there were no detectable irregular antibodies in the donor plasma.[67]

We do keep reminding ourselves regarding clinical significance of the alloantibodies. The Rh blood group is one of the most complex blood groups known among blood group system. D antigen is considered to be the most immunogenic of all antigens and has the potential to cause clinically significant Haemolytic Disease of Foetus and New born (HDFN) and transfusion reactions. Anti-C and anti-E, do not often cause HDFN, and when they do, it is usually mild according to Fung et al study.[68] Anti-P1 and anti-Lewis both are of IgM type and occurs naturally; they are often detected as a weak, room temperature agglutinin. In rare cases, they are reactive at 37° C or shows in vitro haemolysis. Both are of IgM type, do not cross the placenta and do not cause HDFN.[68]

Poole et al discussed that It is often easy to predict clinical significance by evaluating the serology, the tests like IAT, SPRCA do not always distinguish between clinically significant and clinically benign antibodies.[69]

Anstee DJ referred in his study that maybe answers could be found in molecular studies. The development of high-throughput molecular methods means that it may soon be feasible to test large numbers of blood donors for all clinically important blood group polymorphisms.[70] Molecular technology, however, has done little to help us predict the potential clinical importance of an antibody. We still do not understand why some antibodies are hemolytic and clinically significant, whereas others are not. Until we gain more insight into this, the technology of predicting the clinical significance of an antibody will remain, at best, somewhat haphazard.[69]

PERSISTENCE OF ALLOANTIBODIES

Next question that comes to mind is that once the alloantibodies formed in an individual how long will they stay or persist long enough to affect upon transfusion. Only few quantitative data and studies have been presented on this topic. The persistence of alloantibodies should depend upon life of IgM or IgG theoretically.

Roberto Reverberi in his original article, The Persistence of red cell alloantibodies(1989-2008), found 673 antibodies, produced by 525

patients, which had been tested again after the initial detection. The median follow-up was 319 days. The Rate of non-persistence was 37%, corresponding to 251 antibodies, produced by 216 patients.[71]

Non-persisting antibodies were associated with a longer follow-up, more tests after detection, and a lower maximum score (2+ vs. 3+).[71]

Antibody specificity, too, influenced the duration of persistence. Among common antibodies, anti-D was the most long-lived (14% non-persistence); anti-Jk^a the most short-lived (43% non-persistence). [71]

Antibodies detected in the second decade of the study were less persistent ($p<0.001$) and were also weaker (maximum score: 2+ vs. 3+; $p<0.001$). This probably reflects the increased sensitivity of the screening tests over the course of time.[71]

A minority of non-persistent antibodies were detected again after a negative result (intermittently-detected antibodies).[71]

G. Ramsey conducted two studies in this loss of red cell alloantibodies. In Initial study, over time analysis in 160 patients with 209 antibodies was done and retested 1 to 60 months after initial identification. 29% of clinically significant and 72% of clinically insignificant antibodies was not detected on at least one follow-up screening.[72,73]

Anti-Jk^a and anti-C were lost in 59% and 45% of cases, respectively. Patients under 20 years of age may be more likely to lose significant antibodies. Hence, pre-transfusion review of previous records is of vital necessity in Prevention of transfusion reactions.[72]

The other study was a type of follow up. To examine long-term detection, records were reviewed for 44 consecutive patients who were tested more than 5 years after their potentially hemolytic red cell antibodies were first identified.

After 5 to 10 years, 14 (39%) of 36 Rh, Kell, and Duffy system antibodies were not detected on at least one occasion. 22 other such antibodies were sought again after more than 10 years; 10 (45%) were not detected. When re-stimulation by pregnancy was excluded, these rates were 42% and 48% respectively.[73]

Schonewille et al did a retrospective examination of all records of RBC antibodies in the transfusion laboratory computer database from

1978 through 1997. An antibody was regarded as not persistent if, after previous detection, the screening or panel studies became negative for the antibody under study. Anti-D due to RhIg administration was excluded. Among 480 records, 593 antibodies fulfilled the criteria. Median antibody follow-up was 10 months(range1-24). In 137 patients, 153 (26%) antibodies became undetectable over the course of time. After initial negative screening investigations, 310 antibodies were formed. The antibodies that were still detectable had a median follow-up of 7 months(range 1-193). About 25% of all antibodies became undetectable over the course of time.[74]

In prospective study of **Engelfriet et al** showed that 30% to 35% of antibodies that must be considered to be of clinical significance became undetectable in about 1 year and that nearly 50% became undetectable after 10 or more years. Cases are well known in which anti-D can be readily detected in the serum of a D-negative woman 30 years or more after her last pregnancy with a D-positive child.[75]

The Kidd blood group antibodies usually are produced for a short period of time after each antigenic stimulus. **Cox et al** reported an anti-Jk^b that is still demonstrable 16 years after initial detection. In April 1967, a 29-year-old Caucasian woman was first shown to have an anti-Jk^b specificity in her serum. She has never been transfused to the best of her knowledge. This case illustrates an unusual long-term persistence of an anti-Jk^b in a multiparous woman with consistently Jk^b positive fetuses.[76]

Positive DATs are reported in 1 in 1000 to 1 in 14,000 healthy blood donors, and 1 in 100 to 1 in 17 in hospitalized patients without clinical manifestation of hemolysis or hemolytic anemia. Multiple diseases are associated with a positive DAT, not necessarily correlating with the presence of hemolytic anemia, such as sickle cell disease, thalassemia, renal disease, multiple myeloma, autoimmune disorders and HIV/AIDS.[77]

As we have learnt from previous conducted studies that exposure to the RBC carrying an antigen lacking on the RBCs of the recipient can elicit an immune response in some individuals. Therefore, Blood group

antigens are clinically significant in allogeneic blood transfusions, materno-fetal blood group incompatibility, autoimmune hemolytic anemia and organ transplantation.

Our knowledge in alloimmunization of donor population, the causes and effects on recipient of blood is still lacking. The present study is set out to see the prevalence of RBC alloimmunization in healthy donor population.

MATERIAL AND METHODS

This was a prospective study performed on 26772 healthy blood donors who donated for Dayanand Medical College & Hospital, Ludhiana either through camps or Indoor at the blood centre. All the whole blood donors were initially screened for atypical red cell antibodies with Capture-R Ready Screen technology (pooled cells).

The positive cases were repeatedly tested on a new sample. False positives were dropped. True positives were taken up for Bio-Rad 3-cell Panel antibody detection and Bio-Rad 11-cell panel antibody identification. Direct Agglutination test was put up in required cases.

- The initial positive screened blood was quarantined till further testing results.
- Follow up telephonically with the cases was done.
- Any required history of medical, surgical or blood transfusion was reconfirmed.
- For positive cases that did show up for follow up repeat sample was taken and tested.
- Confirmed cases were made rare blood group donor card and explained about frequent interval testing to see if antibodies disappear over time.
- Blood component of identified and confirmed cases was discarded to prevent any future Transfusion reaction.

SOURCE OF DATA

Source used for the study were data collected from blood donations at the department of Immunohematology and Blood transfusion, Dayanand Medical College & Hospital, Ludhiana, Ludhiana.

METHOD OF COLLECTION OF DATA

All the samples from donated blood were tested here.

Inclusion Criterion – Age >18 years, Hb>=12.5, Weight>45 kg, BP-within normal limit (WNL).

Exclusion Criterion – Fever/Cough (last 3 days), Anemia, Diabetic, Heart Disease, Bleeding Tendency, H/O Antibiotics, Allergy, H/O Malaria, Dengue, Epilepsy, Jaundice, Typhoid, Drug addiction, Asthma,

Major blood loss, inking of tattoo in time period of one year, Tuberculosis.

DURATION OF STUDY: Time Period of One Year

OUTCOME: A blood sample which test positive for unexpected antibodies, its blood and blood component were quarantined and discarded to prevent transfusion reactions in the future, thus ensuring safe blood.

STATISTICAL METHOD:

The data was analyzed as follows.

Data were used described in terms of range; mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

OBSERVATIONS AND RESULTS

Red cell antibody screening was conducted on 26772 blood samples of healthy whole blood donors at Dayanand Medical College Hospital, Ludhiana from January 2019 to December 2019. The descriptive statistics are as follows.

Table 1: Distribution among the camp and blood centre donations

Donation	Number of Donations	Number of Screen positive cases of each location	Percentage of cases among total cases
OUTDOOR(CAMP)	6328	16(0.25%)	20.3%
INDOOR	20444	63(0.30%)	79.7%
Total	26772	79(0.30%)	100.0%

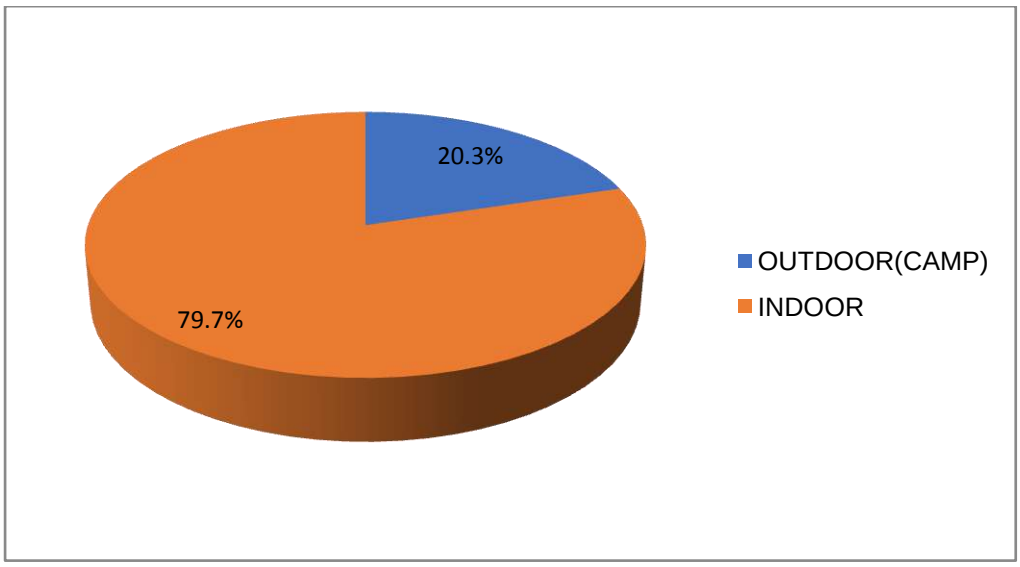


Figure 8: Distribution among the camp and blood centre donations

The above pie chart depicts that maximum collection and maximum cases were seen in Indoor collection 79.7% (63/79*100). We had more indoor donations (including indoor camps) and hence more cases from Indoor as compared to 20.3% (16/79*100) in Camps. Both **locations** have the different atmosphere, setting for taking **history** and time per donor for general physical examination.

Table 2: Gender wise distribution of donors and their percentages

Gender	Total	No. of cases	Percentage of total cases
Female	643	4	5.1%
Male	26129	75	94.9%
Total	26772	79	100.0%

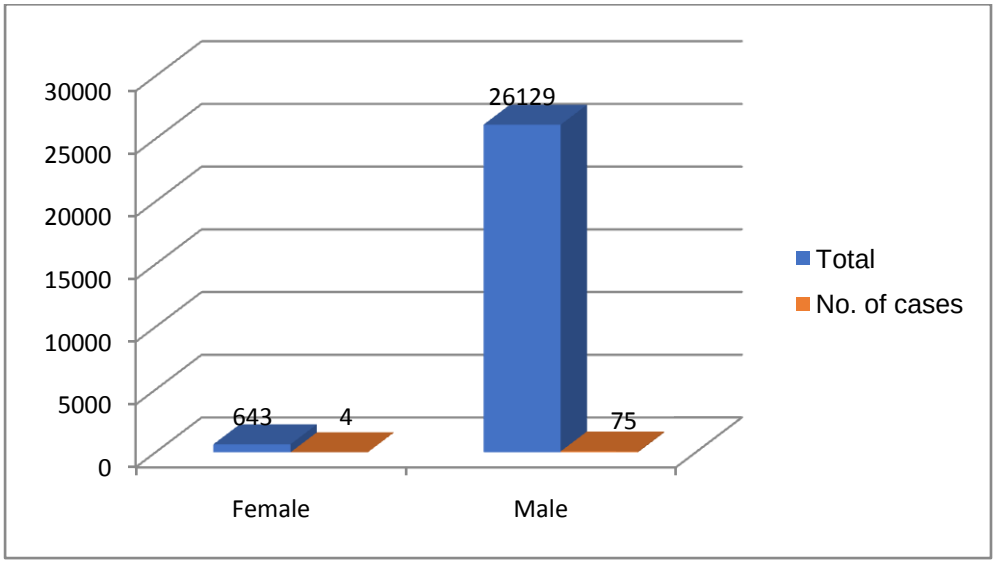


Figure 9: Gender wise distribution of positive cases

In our study males were 94.9% (4/79*100) of the cases and females were only 5.1% (75/79*100). In time period of one year, male donors were 26129(97.5%) while female were 643(2.4%) each gender having 75 and 4 as positive cases respectively. Proportionately female predominance was seen as more cases occurred for lesser number of female donors.

Table 3: Donor Percentage for first time and repeat donation

First/Repeat	No. of cases	Percentage
FIRST	33	41.8%
REPEAT	46	58.2%
Total	79	100.0%

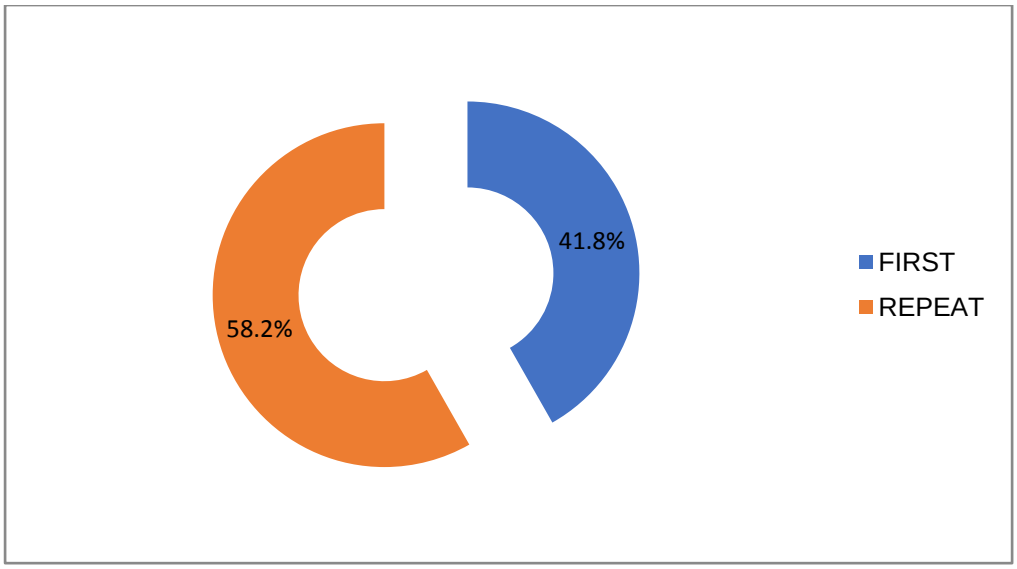


Figure 10: Donor Percentage for first time and repeat donation

It is important for a donor to know about his positive blood screen for allo/auto antibodies. In this study we looked into details whether the donor had been tested prior to this donation. 41.8% (33/79*100) were first time donors while 58.2% (46/79*100) were repeat donors with no knowledge of allo/auto antibodies prior to donation.

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Table 4: Distribution of cases within city and adjoining areas

Donor Regional Division for Punjab and other states	No. of cases	Percentage
Malwa	60	75.9%
Doaba	7	8.9%
Majha	2	2.5%
OTHER States	10	12.7%
Total	79	100.0%

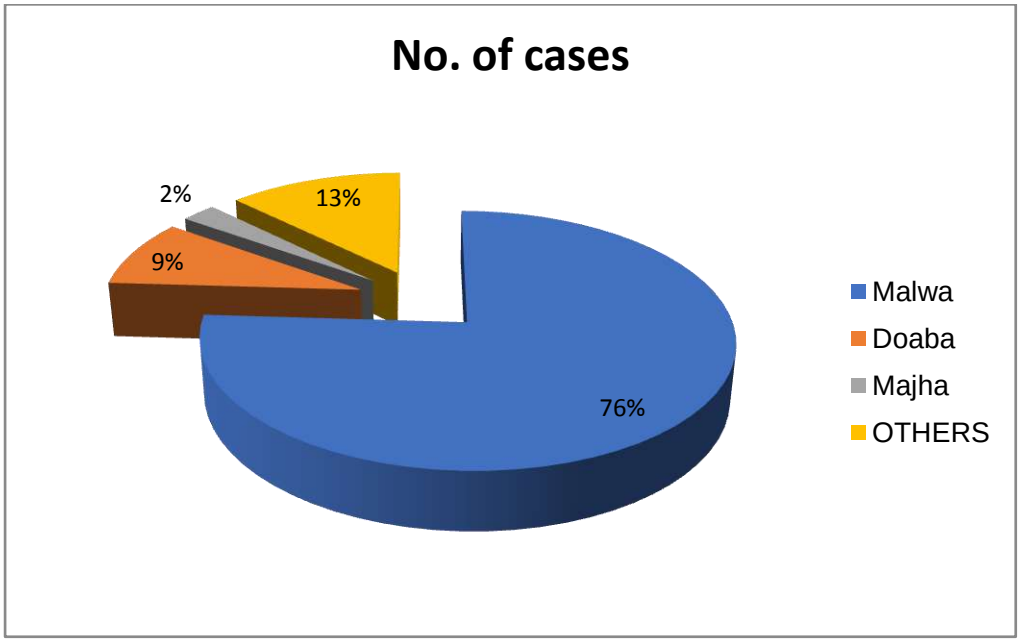

Figure 11: Demographic distribution of cases city and adjoining areas

Our centre receives donations from people mostly from north part of the country. This can be shown clearly in figure 11 that local population of the city played key role in donation. Having maximum cases 60(60/79*100=75.9%) were Malwa cities including Ludhiana where our blood centre is located and others like Firozpur, Faridkot, Moga, Barnala, Bathinda, Sangrur, Patiala and Fatehgarh Sahib.

Table 5: Age wise distribution of positive cases

Age (years)	No. of Positive cases	Percentage
20-30	34	43.0%
31-40	20	25.3%
41-50	19	24.1%
> 50	6	7.6%
Total	79	100.0%

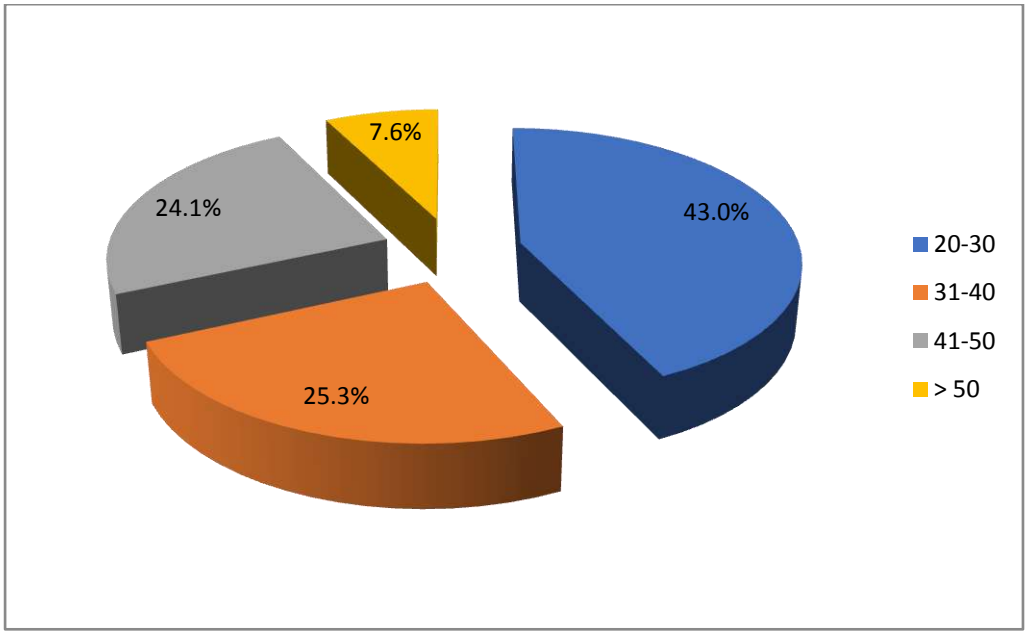

Figure 12: Age wise distribution of positive cases

The age limit for blood donation is 18years to 60years for first time donation and 65 years if it is repeat donation. The maximum range in our study for positive antibody detection was between 20 to 62 years (Mean=35.14yrs) and Standard Deviation (SD) of 9.67 yrs. Weight of donors varied between 56 to 140 kg (Mean=81.59kg) and SD of 15.19kg in positive screen cases. The group with maximum cases of positive screen was from 20-30 yrs (43%), followed by 31-40(25.3%) and 41-50(24.1%).

Table 6: Significant History that may change donor serology

Medical/Surgical History	No. of cases	Percentage
Not Significant	70	88.6%
Medication	6	7.6%
Blood Transfusion	6	7.6%
Surgical	3	3.8%
Rh negative pregnancy, Immunoglobulins	1	1.3%

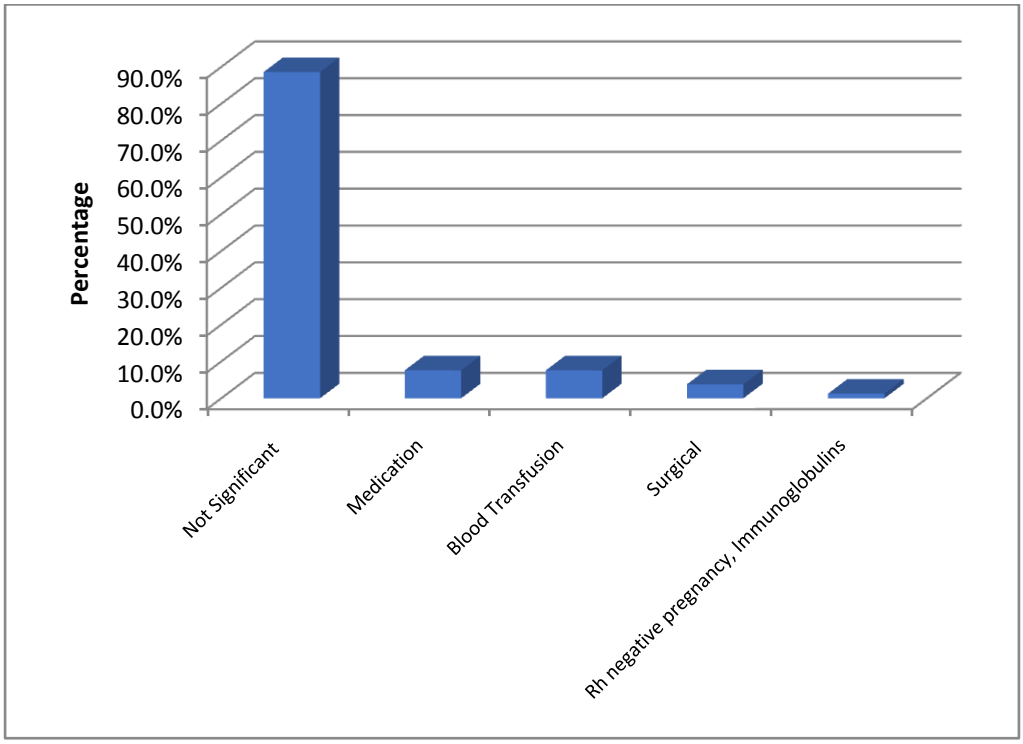

Figure 13: Significant Medical and surgical history of donors

Out of 79, 70 cases (88.6%) had no significant history and were healthy throughout their lives without any major hospital treatment. 6(7.6%) of them had been on regular medication for hypertension or diabetes mellitus. 6(7.6%) had history of previous blood transfusion. Their transfusion took place for treatment during surgery, treatment of RSA or for thrombocytopenia in dengue. 3(3.8%) cases had surgical history. 2 were females with H/O Caesarean section and 1 was male with H/O hernia operation. One female donor had received IVIG following Rh negative pregnancy.

Table 7: Blood group wise distribution of donors and cases

Blood Group	Total donor	Positive cases	Percentage of total cases
B+	9400	24	30.4%
O+	7881	20	25.3%
A+	5345	17	21.5%
AB+	2280	6	7.6%
B-	702	5	6.3%
O-	596	1	1.3%
A-	410	4	5.1%
AB-	158	2	2.5%
Total	26772	79	100.0%

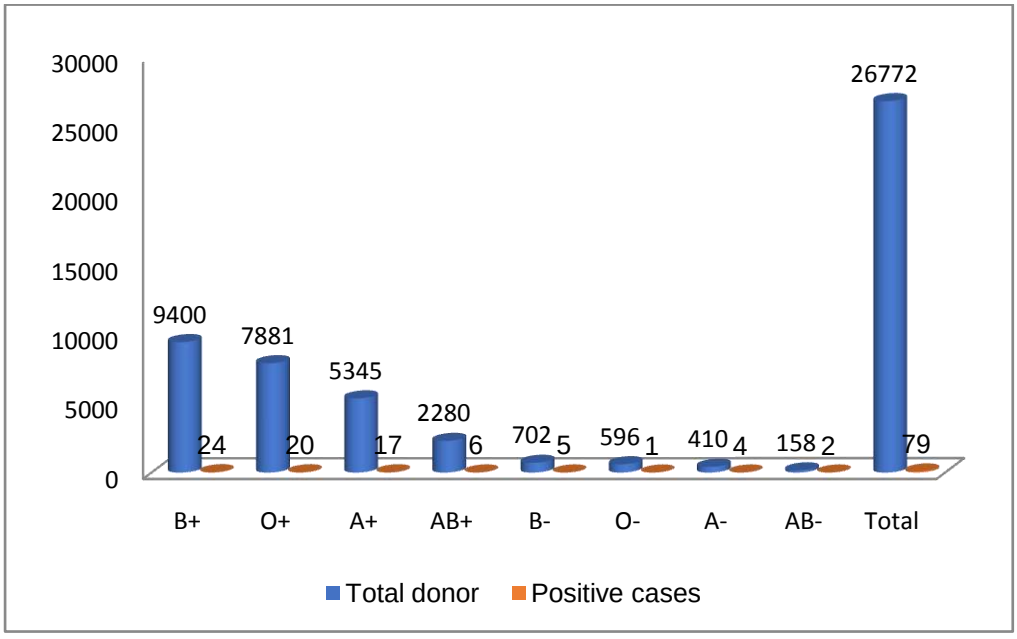

Figure 14: Blood group wise distribution of donors and positive cases

In our region, the blood group frequency consists of B+>O+>A+>AB+>B->O->A->AB-. Weak D testing is not done in routine. The positive cases followed the general profile of B+(30.4%)> O+(25.3%)>A+(21.5%)>AB+(7.6%)>B-(6.3%)>A-(5.1%)>AB-(2.5%)>O-(1.3%) i.e. except A- and AB- blood group cases were more in comparison to O-. Rh positive donors were 24906(93%) while Rh negative donors were 1866(7%).

Table 8: Positive (unexpected IAT reaction) cases percentage

All Donors Screen Test	No. of cases	Percentage
Positive	79	0.3%
Negative	26693	99.7%
Total Donors	26772	100.0%

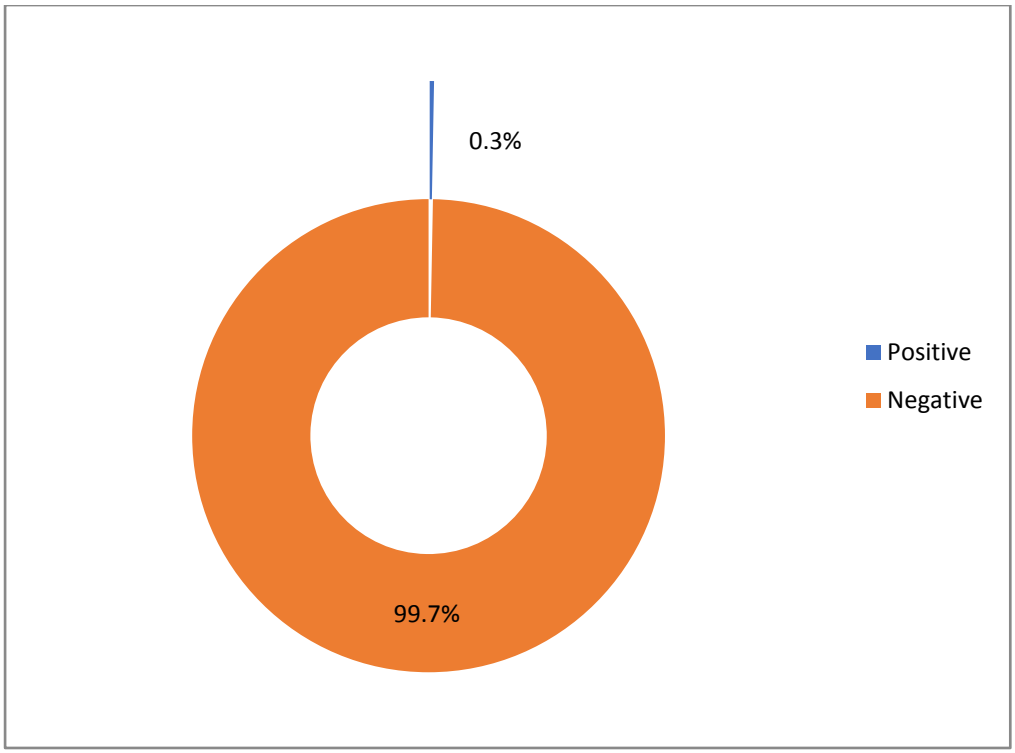

Figure 15: Positive (unexpected IAT reaction) cases percentage in Capture-R screen

Among total donations of 26772 within the time period of one year had 79 positive cases of positive screen, that resulted unexpected reactions percentage to be 0.30%. Remaining 99.7% cases were negative. Cases that showed weak positive reaction less than 1+(Capture-R reaction strength 35/100) or those on repeat testing showed no reaction were taken as negative. Such results can be accounted to high sensitivity of Capture-R screen.

Table 9: Reaction Strength distribution in positive Capture-R screen

Screen with Pooled Cells	No. of cases	Percentage
1+	5	6.3%
2+	60	75.9%
3+	13	16.5%
4+	1	1.3%
Total	79	100.0%

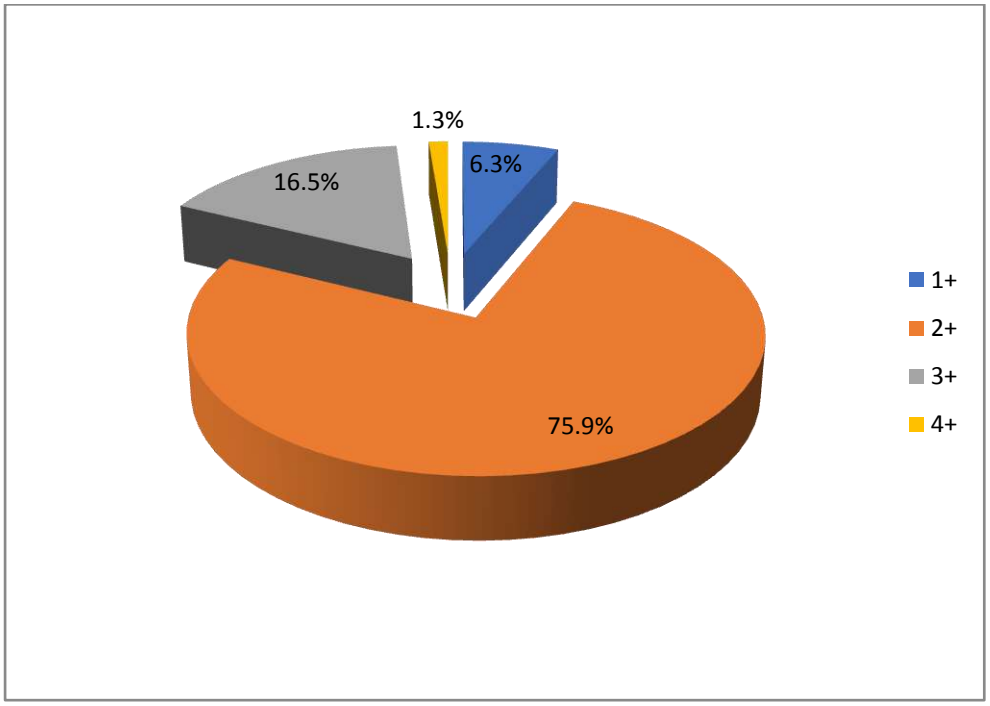

Figure 16: Reaction Strength in Positive Capture R Screen cases

Reaction strength varied for all cases as different antibodies have varying reactivity at the initial tested temperature. We observed that among 79 cases strength varies from 1+ to 4+ grading. 4+ reaction was seen in only 1(1.3%) case and the maximum cases (75.9%) showed 2+ reaction. Remaining cases were either 1+ (6.3%) or 3+(16.5%).

Table 10: Percentage and Reaction Strength of DAT in positive cases

DAT in Positive donors	No. of cases	Percentage
2+	2	2.5%
3+	1	1.3%
Negative	76	96.2%
Total	79	100.0%

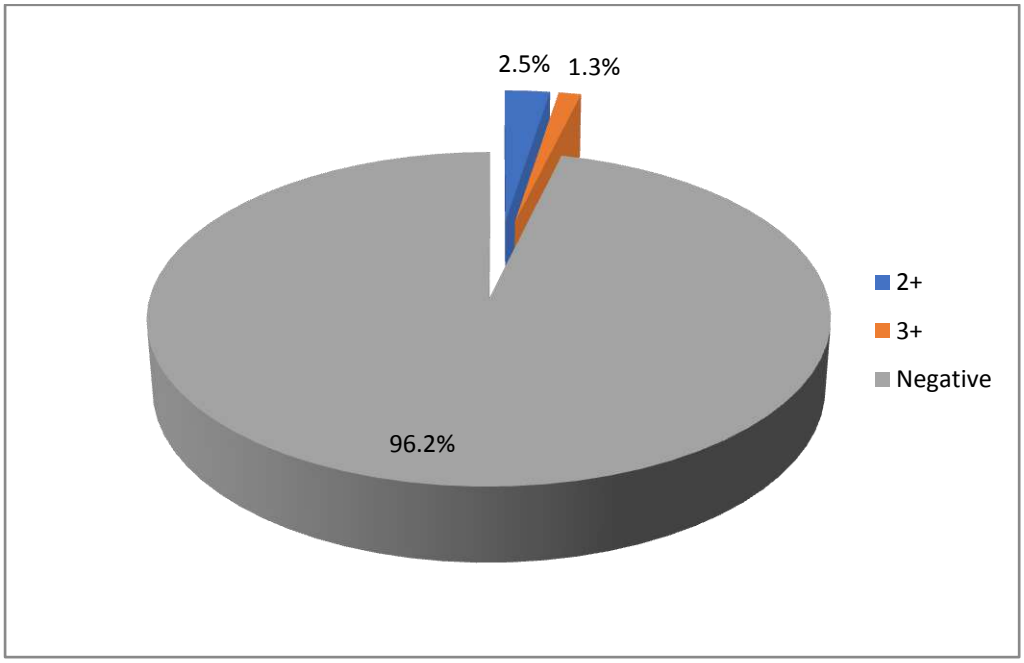

Figure 17: Percentage of DAT positive cases

A DAT was performed as part of the evaluation for unexpected antibodies in positive IAT cases. DAT is not regularly performed in all the donors. In a positive DAT, an eluate may aid in antibody identification. We observed out of 79, DAT was positive in 3 cases. Reaction strength in 2 cases being 2+ (2.5%) and 1 case being 1+(1.3%).

Table 11: Results of Auto-control put up in positive cases

Auto-Control	No. of cases	Percentage
3+	4	5.1%
Negative	75	94.9%
Total	79	100.0%

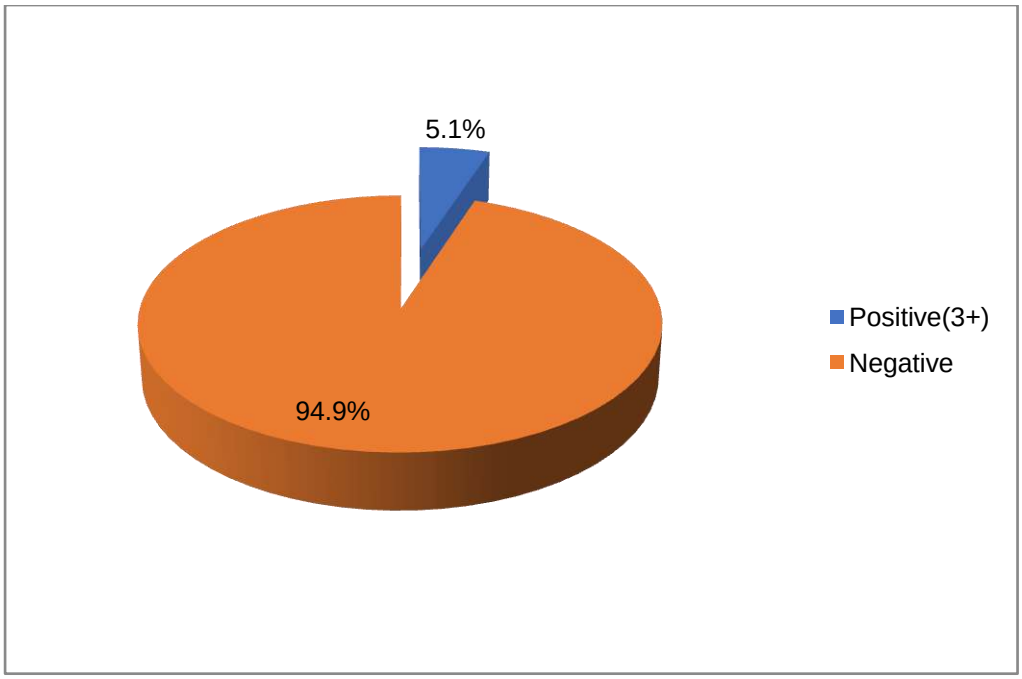

Figure 18: Results of Auto-control put up in positive cases

Auto control was seen in 4 cases among 79 where warm autoantibody was deduced later on. 5.1% is quite a significant percentage comparable to other studies. It is helpful in early diagnosis of cases with predilection to formation of **autoantibodies** or **hematological malignancies** like leukemia.

Table 12: Results of irregular RBC Antibody Identified

Antibody Identification	No. of cases	Percentage
Anti-C	2	2.5%
Anti-C^w	1	1.3%
Anti-D	1	1.3%
Anti-E	3	3.8%
Anti-Jk^a	1	1.3%
Anti-K	1	1.3%
Anti-Le^a + Anti-Le^b	1	1.3%
Anti-Le^b	1	1.3%
Anti-M	28	35.4%
Anti-N	2	2.5%
Anti-P1	5	6.3%
Anti-P1+Le^b	1	1.3%
Anti-S	1	1.3%
Warm AutoAntibody	4	5.1%
Inconclusive	27	34.2%
Total	79	100.0%

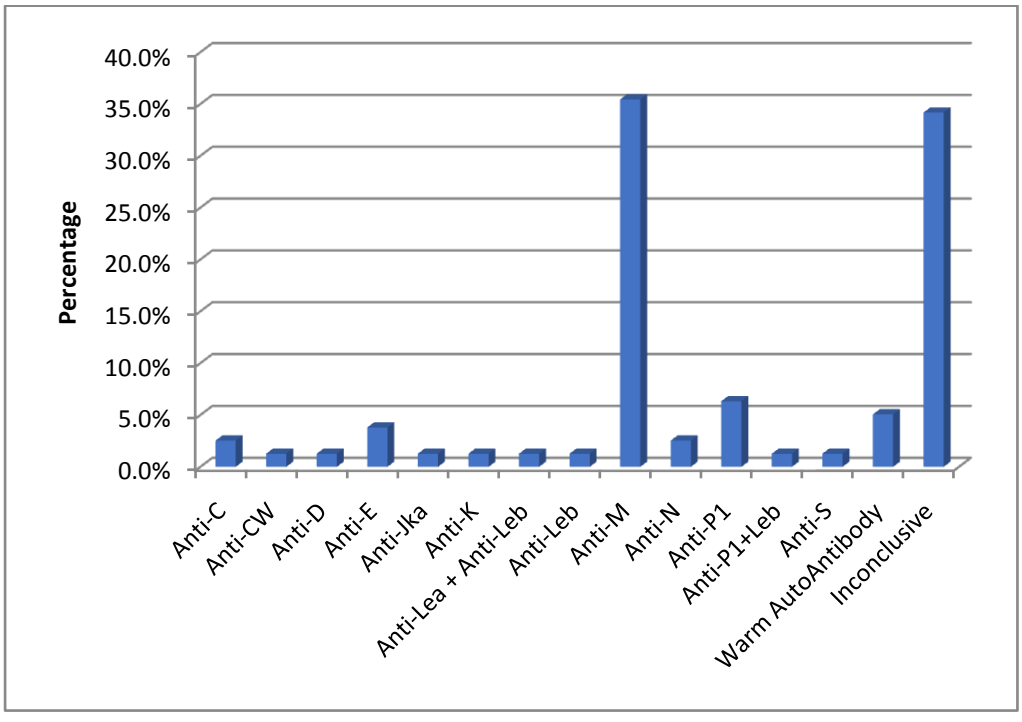

Figure 19: Frequency of antibody identified

As the chart above shows that maximum cases of Anti-M 28(35.4%) were seen, followed by Anti-P1 5(6.3%) plus one in combination with Anti Le^b(7.6%), Anti-E 3(3.8%), Anti-C 2(2.5%), Anti-N 2(2.5%) and one case each of Anti-C^w, Anti-D, Anti-Jk^a, Anti-K, Anti-Le^a + Anti-Le^b, Anti-Le^b, Anti-P1+ Anti-Le^b, Anti-S. Four cases of Warm autoantibodies were seen(5.1%).

Table 13: Thermal amplitude of every Antibody identified percentage

Thermal Amplitude (^{o}C)	No. of cases	Percentage
37 (Warm antibody)	13	16.5%
4 to 22 (Cold antibody)	37	46.8%
4 to 37 (Wide Range)	2	2.5%
Not Determined	27	34.2%
Total	79	100.0%

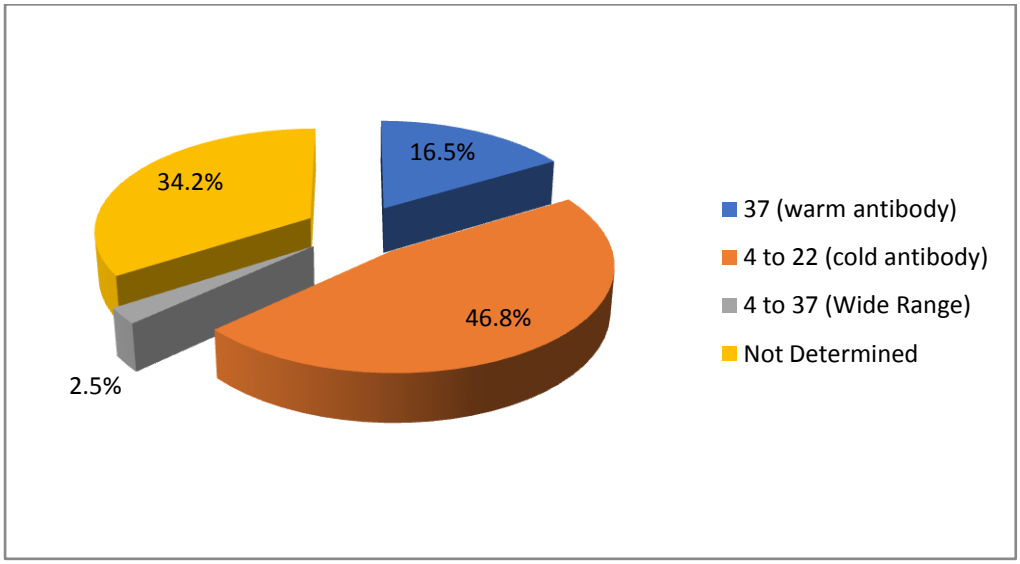

Figure 20: Thermal amplitude of antibodies with their percentage

Maximum antibodies found in our study belonged to cold group which were Anti-M and Anti-P1 and mostly clinically not significant. The thermal amplitude was an observation at which the antibody could be detected and identified.

DISCUSSION

Exposure to the RBC carrying an antigen lacking on the RBCs of the recipient can elicit an immune response in some individuals. Therefore, Blood group antigens are clinically significant in allogeneic blood transfusions, feto-maternal blood group incompatibility, autoimmune hemolytic anemia and Organ transplantation.

Our knowledge in alloimmunization of donor population, the causes and effects on recipient of blood is still lacking. The present study is set out to see the prevalence of RBC alloimmunization in healthy donor population. **Blood grouping and red call antibody testing** though heavily biased towards the prevention and management of the continuing problems of HTR, HDFN and also give advanced warning of possible difficulties about blood supply. **Type and Screen method** goes to great length in supporting blood transfusion services with minimum managed inventory.

This study acts as a guide for all involved in blood transfusion services as well as for the clinicians to treat the topic of transfusion with very delicately due to the presence of alloantibodies in healthy donors. Unless we follow through a case we may never know whether the given alloantibody is capable of causing hemolysis or benign.

The present Study shows incidence similar to the previously conducted studies as quoted in the literature. **The incidence in the present study for irregular RBC alloantibodies is 0.3%**.

Table 14: Prevalence of irregular RBC antibodies in different studies across the world given below.

S. no.	Place	Reference	Year	Samples Tested	Positive Screen	Alloanti-body(%)	Allo with Auto Antibody (%)	Auto Anti-body (%)	Antibodies Found	Nonspecific
1	Seattle	Giblett et al[44]	1977	213254	685	0.32(%)	-	-	Anti-D,E,K	-
2	Kuwait	Ameen et al[47]	2005	179045	-	0.49(%)	-	-	Anti-D,E,K,vLe(a),vLe(b)	-
3	Shaoguan	Zhu et al[59]	2007	15033	42	0.28(%)	-	-	Anti-D, E, E+c,C,Lewis	-
4	India	Pahuja et al[48]	2012	7756	4	0.05(%)	-	1	Anti-C,Le(a)	-
5	South Thailand	Promwong et al[62]	2013	-	-	0.6(%)	-	-	Anti-Le(a),Mi(a), Le(b),M,D	-
6	India	Garg et al[49]	2014	47450	46	0.09(%)	-	6 (0.01%)	Anti-N,M,D	4
7	Iran	Babaei et al[39]	2016	75	6	8	-	-	-	-
8	East Malaysia	Shafini et al[46]	2017	500	4	0.8(%)	-	-	Anti-Mi(a),Le(a)+Le(b)	-
9	India	Kaur et al[56]	2017	6350	7	0.11(%)	-	0.05%	Anti-M,Le,	1
10	Columbia	Garcia et al[61]	2017	60309	-	0.06(%)	-	-	Antibodies from Rh blood group	-
11	Egypt	El-Faramawy et al[43]	2018	200	8	4	-	-	Anti-k,C,M,c,P1	37.50%
12	India	Patidar et al[63]	2018	18112	17	0.09(%)	-	1 (0.01%)	Anti-M,E, Le(a), K, N+Le(a), Cw	1 (unidentified)
13	West Norway	Erikstein et al[64]	2018	55462	-	0.73(%)	-	-	Anti-E,M,K,D,Fy(a)	-
14	India	Makroo et al[38]	2018	82153	227	76(0.09%)	1 (0.001%)	150 (0.18%)	Anti-M and Anti-D	-
15	DMCH, India	Present Study	2019	26772	79 (0.29%)	48(0.18%)	-	4 (0.01%)	Anti-M, P1, D, N, C.Cw,E, K,S,Jk(a), Le(a),Le(b)	27(0.01%)

Comparison of our data with the literature reflected a few differences and few similarities with other studies.

The large variation of prevalence of alloantibodies may be due to the **different screening method** used, and characteristics of the population studied.

In our study the total positive screen percentage was found in 79 cases in total 26772 making it 0.3%. It is comparable to other studies like **Zhu et al.**[59] 0.28%, **Giblett et al.**[44] had reported 0.32% incidence of irregular RBC antibodies in blood donors. However, **Makroo et al**[38] shown prevalence of 0.09%, **Pahuja et al.**[48] 0.05% and similarly **Garg et al.**[49] reported a prevalence of 0.09% among 47,450 whole blood donors.

However in **Giblett et al**.[44] the incidence went down to 0.04% after the donors with any history of blood transfusion or pregnancy were excluded from the analysis making the reports comparable. In our study, 6 donors underwent some form of blood transfusion once in their lifetime. Similarly, **Winters et al.**[45] in 2001 reported 0.89% prevalence among blood donors of Olmsted County, Minnesota that consisted of donors who were previously transfused and pregnant women in their study, which probably explains the higher percentage of alloantibodies. The highest frequency of alloantibodies was identified in blood donors aged between 20 and 30 years in our study. Similar results were reported by **Pahuja et al.**[48] In the present study, we found a predominance of female donors compared to male donors, which is comparable with a study done by several other except **Makroo et al**[38] and **Garg et al.**[49] In another retrospective analytical study of RBC alloimmunization among 179,045 Kuwaiti patients, pregnant women, and allogeneic blood donors was conducted for 1992–2001 by **Ameen et al.**[47] in 2005. Overall incidence of alloimmunization seen in the general population was 0.49% and in blood donors was 2.3%. The incidence among female patients and donors was >3 times higher than among male subjects. In this study, however, the prevalence of irregular RBC alloantibody in males (*n* = 63; 82.90%) was higher than

females (*n* = 13; 17.10%). This finding was consistent with the reports of **Pahuja et al**.[48]

On identification in 79 cases in our study**, 48 (61%) had allo-antibodies** and **4(5.1%) had auto-antibodies** (warm auto antibodies) while **27(34.2%) were inconclusive**. Anti-M (n =28; 35.4%) was the most common antibody identified, followed by anti-P1 (n = 5; 6.3%) plus one in combination with Anti-Le^{b}. In our study, the most frequent alloantibodies identified were from the MNS blood group system followed by P1 blood group system. Anti-M and anti-P1 are generally naturally occurring alloantibody which do not react at 37°C, and are not clinically significant for transfusion but can cause a problem in pre-transfusion testing. It is clinically significant when detected at 37°C, wherein, cross-match compatible antigen negative blood should be given to prevent any hemolytic transfusion reaction. In our units positive for Anti M and Ant-P1, we utilized the Packed red cells while the plasma component units were quarantined.

The Rh blood group is one of the most complex blood groups known among blood group system. D antigen is considered to be the most immunogenic of all antigens and has the potential to cause clinically significant Hemolytic disease of Fetus and New born (HDFN) and transfusion reactions. Anti-C and anti-E do not cause HDFN often but may do so very rarely.

The frequency of anti-D in our study was found to be 1.3%. The donor with anti-D (*n* = 1), was a female from Outdoor Camp with history upon follow-up of Rh negative pregnancy and RhO(D) immunoglobulins. The frequency of minor antigens anti-E and anti-C was found to be 3.8% and 2.5%. In comparison with the other study reported, frequency of anti-D found in our study was low could be because more number of male donors. In our study, antibody screening and identification was performed by SPRCA technique, whereas, they used column agglutination technique. The sensitivity of antibody detection by SPRCA is higher as compared to column agglutination.

Other alloantibodies found in our study are anti-Jk^a (1.3%), anti-Le^a +Anti-Le^b (1.3%), and anti-Le^b (1.3%) plus one in combination with Anti-P1. However, the results were remarkably different from a study done in Chinese blood donors by **Zhu et al.** [59], where anti-Le^b were 7.1% and 2.3%, and no anti-Jk^a was found in their donor population. This difference can be attributed to the heterogeneity of the population, and differences in the prevalence of antigens according to the ethnicity. Anti-Jk^a is of IgG type; hemolytic transfusion reactions are very common because Kidd antibodies are often not detected in pre-transfusion testing as their levels in plasma drop below the detectable level and they show dosage effect. The clinically significant antibodies both the packed red cells and plasma component units were quarantined.

Anti-P1 and anti-Lewis both are of IgM type and occurs naturally; they are often detected as a weak, room temperature agglutinin. In rare cases, they are reactive at 37° C or shows *in vitro* hemolysis. Both are of IgM type, do not cross the placenta and do not cause HDFN. In our study, 4 (4/26772*100=0.01%) had autoantibodies (DAT positive) which is much lower than reported by **Tiwari et al**[58] (0.04%) and **Kaur et al** [56] (0.05%). The samples with suspected autoantibodies were sent to reference laboratory for further testing and confirmation. Similar results were seen in studies conducted by **Garg et al.**[49] 0.01%, **Patidar et al**[63] 0.01% and **Makroo et al**[38] 0.18%. At our centre as per institutional policy, these blood units were discarded and the donors were informed for getting regular medical examination. In **El-Faramawy et al**[43] study frequency of blood groups in their population were A (35.5%), O (32.5%), B (22%), AB (10%), and as regard Rh (97%) had Rh positive and (3%) were Rh negative; while in **Babaei et al**[39] frequency of blood groups were A (52%), O (29.33%), B (14.66%) and AB (4%), Rh+(97.33%) and Rh-(2.67%).

The frequency of the blood groups in our study matched with regional data of gene frequency: B+(30.4%), O+(25.3%), A+(21.5%), AB+(7.6%), B-(6.3%), A-(5.1%), AB-(2.5%) and O-(1.3%), Rh positive donors were 24906(93%) while Rh negative donors were 1866(7%).

Out of all 79 positive screens, 27 cases were put in **inconclusive** results as we could not find the evident serological reason. It could be that solid-phase testing used has increased sensitivity and may detect weak autoantibodies that other method may miss. Such results were also noticed by **El-Faramawy et al**[43] and **Patidar et al.**[63]

There are more important problems to consider, such as developing better laboratory tests for predicting the post-transfusion viability of red blood cells, white blood cells and platelets; improving the efficiency of methods used for producing blood components; educating medical students, house officers, and our colleagues in practice to ensure the proper ordering and handling of blood and its components; and carefully assessing the therapeutic value of such special preparations as white blood cell concentrates, frozen red blood cells and purified plasma proteins.

As professionals pursuing the art and science of blood transfusion, we need to pay full attention to the donors, as well as to the recipients of blood. Donors and volunteers come to our institutions with the expectation that the blood they donate will be used to the best possible advantage. Every resource should be utilized properly and no blood should be left unused or discarded because of some capricious notion. For example, plasma removed from the blood of sensitized donors should be fractionated or used as typing reagents, and the remaining red blood cells issued for transfusion. We have a moral obligation to the donors as they have designated us as the guardians of their blood; we owe it to them to behave accordingly.

SUMMARY AND CONCLUSION

During the course of the study we learnt and reviewed new concepts and came across several challenges. The key points have been listed below:

1. Regular Blood Screening of donated blood for not only infections but for irregular RBC antibodies should be done.
2. High care should be taken in identifying antibody in plasma/serum of positive cases while also informing the donor.
3. The blood component with positive screen should be quarantined and discarded if it could not be cross-matched for a suitable recipient.
4. Antibodies detected in donor serum/plasma would require extras processing e.g- plasma reduction for utilization of whole blood.
5. Extended Phenotype cross-matching is advisable to uncover low and high prevalence antigens and their corresponding antibodies.
6. Judicious use of blood and blood components should be done for transfusion in patients.
7. Ethnic points should be considered and close family members directing blood to their patients should be discouraged to prevent allo-immunization.
8. In-house preparation of screen cells would be a possible solution to buying expensive Antibody Screen Panel cells with limited shelf life.
9. Technical Staff should be to be regularly trained and updated about antibody identification methods; thereby regular seminars and discussions should be conducted.

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KEY TO MASTER CHART

S. No.	Serial No.
M-	Male
F-	Female
0-	nil
PRBC-	Packed Red Blood Cells
RDP-	Random Donor Platelets
RSA-	Road Side Accident
SDP-	Single Donor Platelet Apheresis
P-	Positive
N-	Negative
MF-	Mixed Field
IAT-	Indirect agglutination test
DAT-	Direct agglutination test
H/O-	History of

S. No.	Name Initials	Month of Register	Age (years)	Gender	Weight (kg)	Region Division	First/ Repeat	Donation Location	Blood Group	Medical/ Surgical History	H/O Blood Transfusion	IAT
1	SV	January	38	M	78	Malwa	REPEAT	INDOOR	A+	0	0	2+
2	AB	January	29	M	114	Malwa	REPEAT	INDOOR	O+	0	0	1+
3	SS	January	41	M	92	OTHERS	FIRST	INDOOR	B-	0	0	2+
4	KS	January	33	M	62	Malwa	REPEAT	CAMP	O+	0	0	2+
5	GS	January	40	M	92	OTHERS	FIRST	INDOOR	B+	0	0	2+
6	JS	January	26	M	86	Doaba	FIRST	INDOOR	A+	0	0	2+
7	JK	January	36	M	97	Malwa	FIRST	INDOOR	B-	0	0	2+
8	VJ	February	40	M	75	Malwa	REPEAT	CAMP	B+	0	0	3+
9	GS	February	27	M	68	Malwa	REPEAT	INDOOR	O+	0	0	3+
10	JS	February	36	M	84	Doaba	REPEAT	INDOOR	O+	0	0	3+
11	CK	February	27	M	110	Malwa	REPEAT	INDOOR	B+	0	0	2+
12	SLB	February	62	M	70	Malwa	REPEAT	CAMP	AB-	Antihypertensives	0	2+
13	JS	March	29	M	80	Malwa	REPEAT	CAMP	A+	0	0	2+
14	PK	March	53	M	70	Malwa	REPEAT	CAMP	B+	0	0	2+
15	SMS	March	21	M	60	Malwa	FIRST	CAMP	AB+	0	0	2+
16	MS	March	35	M	72	Malwa	REPEAT	INDOOR	B+	0	0	2+
17	TS	March	35	M	87	Malwa	FIRST	INDOOR	B+	0	Yes more than 10yrs, RSA	2+
18	JS	April	42	M	60	Malwa	FIRST	CAMP	B+	0	0	3+
19	PG	April	30	M	69	Malwa	FIRST	INDOOR	O+	0	0	1+
20	S.	April	31	F	68	Malwa	REPEAT	CAMP	O-	Rh negative preg, Immunoglobulins	0	2+
21	SS	April	28	M	140	Malwa	FIRST	INDOOR	A+	0	Yes more than 2 yrs back, RDP in Dengue	2+
22	NK	April	27	M	78	Malwa	REPEAT	CAMP	B+	0	0	2+
23	GJ	April	42	M	100	Malwa	REPEAT	CAMP	O+	0	0	2+
24	JS	April	41	M	70	Malwa	REPEAT	INDOOR	A+	0	0	2+
25	KS	May	36	M	100	Malwa	REPEAT	INDOOR	O+	0	0	2+
26	RS	May	45	M	94	OTHERS	FIRST	INDOOR	AB+	Antihypertensives	0	2+
27	RS	May	39	M	72	Malwa	FIRST	CAMP	O+	0	0	2+
28	KB	May	30	M	83	Malwa	FIRST	INDOOR	B+	0	0	2+
29	HS	May	26	M	122	OTHERS	FIRST	INDOOR	A+	0	Yes, SDP in Dengue, 3 yrs back	2+
30	NK	May	31	M	90	Doaba	FIRST	INDOOR	B+	0	0	1+
31	KS	May	26	M	70	Malwa	FIRST	INDOOR	B+		0	2+
32	RT	May	20	M	85	Malwa	REPEAT	INDOOR	O+	0	0	2+
33	GS	May	39	M	81	Malwa	REPEAT	INDOOR	AB-	Antihypertensives	0	2+
34	NR	May	35	F	60	Malwa	REPEAT	INDOOR	A+	0	0	2+
35	IS	June	26	M	72	Malwa	FIRST	INDOOR	O+	0	0	2+
36	H.	July	21	M	74	Doaba	REPEAT	INDOOR	B+	0	0	4+
37	GS	July	41	M	85	Malwa	REPEAT	INDOOR	B+	0	0	2+
38	SK	July	43	F	69	Malwa	FIRST	INDOOR	B+	H/O Cesarean section	Yes , 6 yrs back, Ceserean Section	2+
39	HS	July	27	M	75	Malwa	FIRST	INDOOR	A+	0	Yes, at the time of birth	2+
40	HS	July	41	M	81	Malwa	REPEAT	INDOOR	B-	0	0	3+
41	RM	August	43	M	72	Malwa	REPEAT	INDOOR	O+	0	0	2+
42	SS	August	23	M	71	Malwa	FIRST	INDOOR	A+	0	0	2+
43	SLB	August	42	M	90	OTHERS	FIRST	INDOOR	B+	0	0	3+
44	SS	August	33	M	106	Doaba	FIRST	INDOOR	A+	0	0	1+
45	MK	August	42	M	103	Malwa	FIRST	INDOOR	O+	Hernial Operation	Yes, 2 yrs back, PRBC	2+
46	KP	September	26	M	79	Malwa	FIRST	INDOOR	A+	0	0	2+
47	DN	September	30	F	69	Malwa	REPEAT	INDOOR	O+	H/O Cesarean section	0	2+
48	D.	September	42	M	79	Malwa	REPEAT	INDOOR	O+	0	0	2+
49	MS	September	42	M	63	Malwa	FIRST	CAMP	A-	0	0	2+
50	RS	September	34	M	90	Malwa	REPEAT	INDOOR	AB+	0	0	2+
51	RM	September	62	M	74	Malwa	REPEAT	CAMP	A+	Antihypertensives, Antidiabetic	0	2+
52	MS	September	47	M	62	Malwa	REPEAT	INDOOR	AB+	0	0	2+
53	SK	September	44	M	77	OTHERS	REPEAT	INDOOR	O+	0	0	2+
54	GS	September	23	M	92	Malwa	REPEAT	INDOOR	O+	0	0	2+
55	LK	September	29	M	103	Doaba	REPEAT	INDOOR	B+	0	0	3+
56	SK	October	27	M	79	Doaba	REPEAT	INDOOR	O+	0	0	2+
57	SG	October	38	M	75	OTHERS	FIRST	INDOOR	A+	0	0	3+
58	DS	October	24	M	65	Malwa	FIRST	INDOOR	B+	0	0	2+
59	HS	October	27	M	75	OTHERS	REPEAT	INDOOR	A+	0	0	2+
60	UG	October	30	M	88	Malwa	FIRST	INDOOR	A+	0	0	1+
61	B.	October	29	M	70	Malwa	FIRST	INDOOR	B+	0	0	3+
62	AS	November	38	M	78	Malwa	REPEAT	INDOOR	B+	0	0	2+
63	SS	November	28	M	56	Malwa	FIRST	INDOOR	A-	0	0	2+
64	VJ	November	36	M	85	Malwa	REPEAT	INDOOR	B+	0	0	2+
65	SK	November	44	M	97	OTHERS	REPEAT	CAMP	O+	0	0	2+
66	SS	November	28	M	87	Malwa	REPEAT	INDOOR	B+	0	0	2+
67	JS	November	56	M	75	OTHERS	REPEAT	INDOOR	A+	Antihypertensive, Antidiabetic	0	2+
68	M.	November	23	M	90	Malwa	REPEAT	CAMP	B-	0	0	2+
69	SG	November	46	M	66	Malwa	REPEAT	CAMP	B+	0	0	2+
70	JS	November	25	M	84	Majha	FIRST	INDOOR	A+	0	0	2+
71	RS	November	25	M	77	Malwa	FIRST	INDOOR	AB+	0	0	2+
72	RS	November	58	M	97	Malwa	REPEAT	INDOOR	B+	0	0	2+
73	IS	December	51	M	94	Malwa	REPEAT	INDOOR	O+	0	0	2+
74	MS	December	26	M	60	Malwa	REPEAT	INDOOR	O+	0	0	3+
75	RK	December	50	M	74	Majha	FIRST	INDOOR	B+	Antihypertensive	0	2+
76	HS	December	36	M	90	Malwa	FIRST	INDOOR	AB+	0	0	3+
77	AS	December	41	M	75	Malwa	REPEAT	INDOOR	A-	0	0	2+
78	GS	December	26	M	84	Malwa	REPEAT	INDOOR	A-	0	0	3+
79	AS	December	27	M	100	Malwa	REPEAT	INDOOR	B-	0	0	3+

S. No.	Name Initials	DAT	Auto Control	Antibody Screen I cell	Antibody Screen II cell	Antibody Screen III cell	Antibody Identification	Antibody Type	Thermal Amplitude (°C)
1	SV	N	N	N	N	2+	Anti-M	IgM	4 to 25
2	AB	N	N	MF	N	MF	Inconclusive	NILL	NILL
3	SS	N	N	N	N	2+	Anti-M	IgM	4 to 25
4	KS	N	N	N	N	2+	Anti-M	IgM	4 to 25
5	GS	N	N	MF	N	N	Inconclusive	NILL	NILL
6	JS	N	N	N	N	MF	Inconclusive	NILL	NILL
7	JK	N	N	N	N	2+	Anti-M	IgM	4 to 25
8	VJ	N	N	N	N	3+	Anti-M	IgM	4 to 25
9	GS	N	N	N	N	3+	Anti-M	IgM	4 to 25
10	JS	N	N	N	N	3+	Anti-M	IgM	4 to 25
11	CK	N	N	1+	N	N	Inconclusive	NILL	NILL
12	SLB	N	N	N	1+	N	Anti-Le^b	IgM	4 to 25
13	JS	N	N	N	N	2+	Anti-M	IgM	4 to 25
14	PK	2+	3+	N	N	N	Warm AutoAntibody	IgG	37
15	SMS	N	N	N	MF	N	Inconclusive	NILL	NILL
16	MS	N	N	N	N	2+	Ant-M	IgM	4 to 25
17	TS	N	N	3+	1+	N	Anti-Jk^a	IgG	37
18	JS	N	N	N	N	3+	Anti-M	IgM	4 to 22
19	PG	N	N	N	N	1+	Inconclusive	NILL	NILL
20	S.	N	N	3+	3+	3+	Anti-D	IgG	37
21	SS	N	N	N	1+	N	Anti-K	IgG	37
22	NK	N	N	1+	1+	N	Anti-P1	IgM	4 to 25
23	GJ	N	N	1+	1+	N	Anti-P1	IgM	4 to 25
24	JS	N	N	N	N	2+	Anti-M	IgM	4 to 22
25	KS	N	N	N	N	N	Inconclusive	NILL	NILL
26	RS	N	N	N	N	1+	Ant-M	IgM	4 to 22
27	RS	N	N	2+	N	N	Anti-C^w	IgG	37
28	KB	N	N	N	N	MF	Inconclusive	NILL	NILL
29	HS	N	N	N	2+	N	Anti-E	IgG	37
30	NK	N	N	MF	N	N	Inconclusive	NILL	NILL
31	KS	N	N	N	1+	N	Inconclusive	NILL	NILL
32	RT	N	N	1+	1+	1+	Anti-P1	IgM	4 to 25
33	GS	N	N	2+	1+	1+	Anti-N	IgG, IgM	4 to 37
34	NR	N	N	N	1+	N	Anti-Le^a + Anti-Le^b	IgM	4 to 25
35	IS	N	N	MF	MF	N	Inconclusive	NILL	NILL
36	H.	N	N	3+	N	N	Anti-C	IgG	4 to 37
37	GS	N	N	N	MF	N	Inconclusive	NILL	NILL
38	SK	N	N	N	2+	N	Anti-E	IgG	37
39	HS	N	N	1+	N	1+	Anti-e	IgG	37
40	HS	N	N	3+	3+	3+	Anti-N	IgM	4 to 25
41	RM	N	N	N	N	N	Inconclusive	NILL	NILL
42	SS	N	N	N	N	N	Inconclusive	NILL	NILL
43	SLB	2+	3+	N	N	N	Warm AutoAntibody	IgG	37
44	SS	N	N	MF	MF	N	Inconclusive	NILL	NILL
45	MK	3+	3+	2+	2+	2+	Warm AutoAntibody	IgG	37
46	KP	N	N	N	N	N	Inconclusive	NILL	NILL
47	DN	N	N	3+	N	N	Anti-C	IgG	37
48	D.	N	N	N	MF	N	Inconclusive	NILL	NILL
49	MS	N	N	1+	1+	N	Anti-M	IgM	4 to 25
50	RS	N	N	N	MF	N	Inconclusive	NILL	NILL
51	RM	N	N	MF	N	N	Inconclusive	NILL	NILL
52	MS	N	N	2+	1+	N	Anti-P1+Le^b	IgM	4 to 25
53	SK	N	N	MF	MF	N	Inconclusive	NILL	NILL
54	GS	N	N	1+	N	1+	Anti-P1	IgM	4 to 25
55	LK	N	N	3+	N	N	Anti-S	IgG	37
56	SK	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
57	SG	N	N	3+	1+	1+	Anti-M	IgM	4 to 22
58	DS	N	N	N	N	N	Inconclusive	NILL	NILL
59	HS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
60	UG	N	N	MF	N	N	Inconclusive	NILL	NILL
61	B.	N	N	3+	1+	1+	Anti-M	IgM	4 to 22
62	AS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
63	SS	N	N	N	N	N	Inconclusive	NILL	NILL
64	VJ	N	N	2+	1+	1+	Ant-M	IgM	4 to 22
65	SK	N	N	N	N	N	Inconclusive	NILL	NILL
66	SS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
67	JS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
68	M.	N	N	N	N	N	Inconclusive	NILL	NILL
69	SG	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
70	JS	N	N	MF	N	N	Inconclusive	NILL	NILL
71	RS	N	N	N	2+	2+	Anti-P1	IgM	4 to 22
72	RS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
73	IS	N	N	2+	1+	1+	Anti-M	IgM	4 to 22
74	MS	N	N	3+	1+	2+	Anti-M	IgM	4 to 22
75	RK	2+	3+	2+	3+	2+	Warm AutoAntibody	IgG	37
76	HS	N	N	3+	1+	1+	Anti-M	IgM	4 to 22
77	AS	N	N	N	N	N	Inconclusive	NILL	NILL
78	GS	N	N	3+	1+	1+	Anti-M	IgM	4 to 22
79	AS	N	N	3+	1+	1+	Anti-M	IgM	4 to 22

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The Principal,
DMC, Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.05.2019 has approved the plan of thesis of **Bableen Kaur Ghotra**, a student of your college for **MD Immuno Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis

To study the frequency and pattern of irregular rbc alloantibodies in blood donors

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The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF MEDICINE

in

IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

AUGUST, 2018

DR. BABLEEN KAUR GHOTRA

DEPARTMENT OF IMMUNOHAEMATOLOGY AND BLOOD TRANSFUSION

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LUDHIANA, PUNJAB-141001

ABSTRACT OF PLAN OF THESIS

Title	TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS
For the degree of	MD IMMUNOHAEMATOLOGY AND BLOOD TRANSFUSION
Name of the Candidate	Dr. Bableen Kaur Ghotra
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Co-supervisor	Dr. Rajesh Kumar, Professor, Department of IHBT
Co-supervisor	Dr. Sonia Gupta, Associate Professor, Department of IHBT
Institution	DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA
University	BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

Introduction: Provision of safe blood do not just imply for testing for infectious marker, but also for protection from haemolytic transfusion reactions resulting from alloimmunization against red cell antigen. Ever increasing efforts are put in procuring safe blood which has led to incorporation of regular screening protocols for detection of unexpected immune antibodies at various centres across the globe.

Aim of Study: The present study aims to screen antibodies and its identification in blood donors and to provide blood that lacks corresponding antigen to the patient.

Materials & Methods: All the donor samples will be screened for irregular red cell antibodies from January - December 2019. Antibody screening will be performed by solid phase antibody detection method using Capture–R ready screen (pooled cells) and positive samples will be further confirmed using 3 cell and 11 cell panel.

Clinical Significance: Antibodies against red cells can be found in blood donors, detection of which adds a layer of safety in transfusion.

Keywords: Antibody, Antigen, Alloantibody identification, alloantibody screening, donor screening, HTR.

Bableen (Signature) Candidate

(Signature) Co-supervisor

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(Signature) Supervisor

(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS"** exist in the Department of Immunohematology And Blood Transfusion, Dayanand Medical College & Hospital, and Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate himself/herself under our supervision and guidance.

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APPROVAL PROFORMA
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Department	IMMUNOHAEMATOLOGY AND BLOOD TRANSFUSION
Topic of Thesis	To study the Frequency and Pattern of Irregular RBC AlloAntibodies in Blood Donors
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2. HOD Surgery	2.HOD Pathology
3. HOD Pathology	3.HOD Pharmacology
4. HOD Medicine	4.HOD SPM
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6. Vice Principal	6.HOD Surgery
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Research committee

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Approved: Yes/No

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Ethics Committee

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3	**COURSE OF STUDY AND SUBJECT**	MD in Immunohematology and Blood Transfusion
4	**DATE OF ADMISSION OF COURSE**	10th MAY 2018
5	**TITLE OF THE TOPIC**	To study the Frequency and Pattern of Irregular RBC AlloAntibodies in Blood Donors

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Immunoglobulins, or antibodies, have special significance for transfusion medicine, because antigens present on transfused cells may cause reactions in the recipient and complicate therapy. Majority of blood bank testing is focused on the prevention, detection, and identification of blood group antibodies and on the typing of RBC antigens.

A red cell antigen phenotype and/or genotype is detected prior to transfusion with the aim of identifying absent red cell antigens in order to provide antigen-negative red cells for transfusion. It's done as soon as possible before the first transfusion.

The focus of antibody detection methods is on "irregular" or "unexpected" antibodies, as opposed to the "expected" antibodies of the ABO system. The unexpected antibodies of primary importance are the immune alloantibodies, which are produced in response to red blood cell (RBC) stimulation through transfusion, transplantation, or pregnancy. Other unexpected antibodies may be "naturally occurring" (i.e., produced without RBC stimulation). Naturally occurring antibodies may form as a result of exposure to environmental sources, such as pollen, fungus, and bacteria, which have structures similar to some RBC antigens.

Antibodies produced in one individual and then transmitted to another via plasma-containing blood components or derivatives such as intravenous immunoglobulin (IVIG) are known as passively acquired antibodies, a third

category of unexpected antibody. The presence of naturally occurring and passive antibodies may complicate the detection and identification of immune, clinically significant antibodies.

Clinically significant antibodies are those that cause decreased survival of RBCs possessing the target antigen. These antibodies are typically IgG antibodies that react at 37°C or in the antihuman globulin (AHG) phase of the indirect anti-globulin test.

Common antigens causing alloimmunization are: D, E, e, C, c, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s, M, N, Le^a, and Le^b. Red cell antibody anti-A and anti-B are the naturally occurring antibodies that are found in the human serum. All other antibodies are called "irregular red cell antibodies." There are two types of irregular red cell antibodies: - alloantibodies and autoantibodies. Alloantibody is produced against the antigen that is lacking, whereas autoantibody is produced to an antigen that is present. Autoantibodies may also complicate the detection of clinically significant antibodies. Autoantibodies are directed against antigens expressed on one's own RBCs. Because they react with all RBCs tested, autoantibodies may mask the presence of clinically significant alloantibodies.

After detection, an antibody identification panel is performed to determine the specificity of the antibody (or antibodies) present. Once identified, the antibody's clinical significance can be ascertained and the appropriate transfusion considerations put into place.

Only a small percentage of the population (between 0.2% and 2%) has detectable RBC antibodies. However, certain populations require careful

screening. The AABB's Standards for Blood Banks and Transfusion Services requires the use of an antibody screen to detect clinically significant antibodies in both the blood donor and the intended recipient as part of pre-transfusion compatibility testing. The donors may be used as a source of antigen-typing sera and antigen-negative RBC units. An antibody screen may be included when evaluating the compatibility of hematopoietic progenitor cell (HPC) and bone marrow donors with the intended transplant recipient. Antibodies detected in these donors may indicate that additional processing steps are required to reduce the plasma in the product. An antibody screen is included in standard prenatal testing for obstetric patients to evaluate the risk of HDFN in the fetus and to assess the mother's candidacy for Rh-immune globulin (RHIG) prophylaxis.

The National Blood Policy, India, 2007 has laid down the guidelines for the screening of donated blood for the presence of irregular red cell antibodies. The incidence of transfusion reactions due to irregular red cell antibodies in donor blood is rarely seen.[1] However, the presence of such antibodies can occasionally cause severe transfusion reactions if a large amount of plasma or whole blood is transfused as in the cases of massive transfusions or in pediatric population. Procurement of safe blood for transfusion includes not just testing for infectious markers but also protection from Haemolytic Transfusion Reaction (HTR) resulting from alloimmunization. Ever increasing effort at improving blood safety have led to incorporation of Regular Screening Protocol for detection of unexpected Antibody.

6.2 REVIEW OF LITERATURE

Provision of safe blood do not just imply for testing for infectious marker, but also for protection from haemolytic transfusion reactions resulting from alloimmunization against red cell antigen. Ever increasing efforts are put in procuring safe blood which has led to incorporation of regular screening protocols for detection of unexpected immune antibodies at various centres across the globe.

A four-year survey of the incidence of iso-immune antibodies in a volunteer donor population was reported by Myhre et al [2] and average of 0.34% of all units of blood was found yearly. Antibodies were found in 0.56% of first time donors. Several studies have reported that the rate of alloimmunization in blood donors varies from 0.32% to 2.4%.[3,4] This large variation may be due to the different screening method used, and characteristics of the population studied.

The objective of pre-transfusion testing is to ensure that enough red cells and components will survive when transfused, or in other words, to transfuse a blood component to a patient that will provide maximum benefit while causing less harm.

Giblett et al [3] had reported 0.32% incidence of irregular RBC antibodies in blood donors. However, the incidence went down to 0.04% after the donors with any history of blood transfusion or pregnancy were excluded from the analysis making the reports comparable. Similarly, Winters et al [4] in 2001 reported 0.89% prevalence among blood donors of Olmsted county, Minnesota that consisted of

donors who were previously transfused and pregnant women in their study, which probably explains the higher percentage of alloantibodies.

Prevalence of positive antibody screen in Kuwait was 0.49%. The five most frequently identified alloantibodies were anti-D (27.3%), anti-E (18.5%), anti-K (15.6%), anti-Le(a) (8.7%), and anti-Le(b) (6.6%). Alloimmunization frequency was greater among Kuwaiti female than male patients in the ratio of 3.2 F to 1 M. Anti-D ($p<0.001$), anti-E ($p=0.05$), anti-K ($p=0.04$) were also more frequently identified alloantibody among Kuwaiti females. Retrospective analysis of RBC alloimmunization among 179,045 Kuwaiti patients, pregnant women, and allogeneic blood donors was conducted by Ameen et al.[5]

Screening of 15,033 random donor antibodies in Shao-guan area under Zhu et al[6] by screening cells, poly-brene and anti-globulin tests and in results irregular antibodies were found in 42 samples. The frequency of irregular antibodies in female was found to be higher in male populace and Rh blood group antibodies such as anti-D, anti-E, and anti-C were common (47.6%).

Studies on the frequency of red cell alloantibody formation have shown that approximately 2-3% of patients exposed to red cell antigens will form alloantibodies.[7] Alloimmunization incidence among the general population was 0.49% and in blood donors was 2.3%. The incidence of alloimmunization among female patients and donors was >3 times higher than among male subjects. Therefore, it's the need of hour to screen them before transfusing blood.

While considering alloimmunization in donors, one may also look at its frequency in case of patients for comparison. A study done on blood of poly-transfused

patients has shown prevalence of alloantibody specificity in the order of: Anti-E>anti-D>anti-K>anti-C>anti-Di^a>anti-c>anti-Jk^a>anti-S in total of 227. The prevalence of autoimmunization was 20% in Cruz et al.[8]

In the study conducted by Pahuja et al[9] among 7756 screened donors, 7648 donors were males (98.6%) and 108 were females (1.4%). The maximum number of donors belonged to age group of 26–30 years. A total of four donors showed presence of alloantibodies in their serum (0.05%). Anti-Jk^a is of IgG type; haemolytic transfusion reactions are very common because Kidd antibodies are often not detected in pre-transfusion testing as their levels in plasma drop below the detectable level and they show dosage effect.[9]

Irregular alloantibodies/autoantibodies can be encountered in healthy blood donors who are either transfused previously or in multiparous females.[10] Female dominance among donors was observed in study done by Garcia et al.[11]

Overall prevalence of alloantibodies in both patients and donors when using conventional tube techniques was 0.7%; for patients only was 0.9% and donors 0.6% in Promwong et al.[12] Frequent antibodies detected in both groups were anti-Le(a), anti-Mi^a, anti-Le(b), anti-P1 and anti-E. Alloantibodies detected via CAT were found in 0.8% of patients and 0.13% of donors with the five most common alloantibodies being anti-Mi^a, anti-E, anti-Le(a), anti-c and anti-Le(b). Similarly, common alloantibody specificities in donors were anti-Le(a), anti-Mi^a, anti-Le(b), anti-M and anti-D in the Promwong study.[12]

In the study done by Makroo et al[13] on 49,077 patients, screened for the presence of unexpected antibodies. Antibody screening was positive in 403

patients (0.82%). Out of which only in 164 patients autoantibodies were identified, 27 revealed autoantibodies with one or more underlying alloantibodies, while 212 patients had only alloantibody/ies in their serum. The overall alloimmunization rate was 0.49 per cent. Antibodies against the Rh blood group system were most frequent (64.1%), the most common alloantibody identified being anti E (37.2%), followed by anti D (19.2%).

The Rh blood group is one of the most complex blood groups known among blood group system. D antigen is considered to be the most immunogenic of all antigens and has the potential to cause clinically significant Haemolytic Disease of Foetus and New born (HDFN) and transfusion reactions. Anti-C and anti-E, do not often cause HDFN, and when they do, it is usually mild according to Fung et al study.[14]

Anti-P1 and anti-Lewis both are of IgM type and occurs naturally; they are often detected as a weak, room temperature agglutinin. In rare cases, they are reactive at 37° C or shows in vitro haemolysis. Both are of IgM type, do not cross the placenta and do not cause HDFN.[14]

A study done by Garg et al[15] that included total of 47,450 donors out of which 46 donors showed presence of alloantibodies in their serum yielding a prevalence of 0.09%. Most frequent alloantibodies identified were of MNS blood group system.

Cumulative incidence of irregular antibodies amongst patients (n=32,560) and donors (n=31,367) were 0.12 and 0.009%, respectively. Commonest antibodies were from Rh system with anti-D being the most common antibody type (14/40;

35%) while in donors it was the MNS system with anti M (2/3) being the most common in the study done by Tiwari et al.[16]

In Thakur et al[17] total 4573 blood donors, 88.2% were males. Most of the donors (77.6%) were in 18-25 years of age group. The commonest ABO blood group present was B (36.1%) followed by O (29.1%), A (24.8%) and AB (10%) in blood donors; while in Rhesus system, 95.0% donors were Rh-positive and 5.0% donors were Rh negative. In this study there were no detectable irregular antibodies in the donor plasma.

The results showed in Garg et al study[15] showed statistically higher prevalence of RBC alloantibodies in females than in males. It is comparable to recently conducted study done on donors by Makroo et al[18], in which they found a predominance of male donors compared to female donors. The overall prevalence of irregular RBC alloantibodies was 0.09% with anti-M, anti-D being the most frequently identified alloantibodies in blood donors in most recent study.[18]

Red cell antibody screening of the donors is a simple test, adds a layer of safety in transfusion and reduces the need for minor cross matching. In addition, it's recommended that in cases where the antibody is found in blood donor, they should be informed, so that in future if they require any transfusion they can inform the blood bank prior.

6.3 AIMS AND OBJECTIVES OF THE STUDY

- The present study aims to detect the antibody and its Identification in Blood Donors.
- To provide blood that lacks the corresponding antigen to the patient.

7.0 MATERIALS AND METHODS

All the donor samples will be screened for irregular red cell antibodies from January - December 2019. Antibody screening will be performed by solid phase antibody detection method using Capture–R ready screen (pooled cells) and positive samples will be further confirmed using 3 cell and 11 cell panel.

7.1 SOURCE OF DATA

Source used for the study will be the data collected from blood donations at the department of Immunohematology and Blood transfusion, DMCH, Ludhiana.

7.2 METHOD OF COLLECTION OF DATA

- All the samples from donated blood shall be tested here.

Inclusion Criterion – Age >18 years, Hb>=12.5, Weight>45 kg, BP- WNL.

Exclusion Criterion – Fever/Cough (last 3 days), Anaemia, Diabetic, Heart Disease, Bleeding Tendency, H/O Antibiotics, Allergy, H/O Malaria, Dengue, Epilepsy, Jaundice, Typhoid, Drug addiction, Asthma, Major blood loss, inking of tattoo in time period of one year, Tuberculosis.

DURATION OF STUDY

Time Period of One year

OUTCOME

A blood sample which test positive for unexpected antibodies, its blood and blood component should be quarantine and discarded to prevent transfusion reactions in the future, thus ensuring safe blood.

STATISTICAL ANALYSIS

Data will be described in terms of range; mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

No

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes

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PERFORMA FOR BLOOD DONATION

SR. NO.

Demographic Information of Donor

Blood Group -

Donor ID - Voluntary/Replacement Volume - 350/450ml

Name - Father's/Husband's name -

Age/Gender -

Address -

Phone No. Occupation -

First Time/Repeat donation -

Any Discomfort during or after the donation? -

Present/Past History

Fever/Cough (last 3 days)	Jaundice, Malaria, Dengue, Typhoid (last 1 year)	
Unexplained Weight Loss	Tuberculosis	Bleeding Tendency
Drug Addiction	H/o Medication	H/o Asthma
Hypertension	Diabetic	H/o Hospital stay
Heart Disease	H/o Minor/Major Surgery	
Kidney Disease	H/o Tattooing/Acupuncture (last 1 year)	
Epilepsy/Fits	H/o Dental Extraction	
H/o Allergies	H/o recent Vaccination/ Immunoglobulins	
H/o Dog Bite (last 1 year)	H/o Blood Transfusion	
H/o Sexually Transmitted disease		

Signature of Candidate

Signature of Supervisor

Antibody Screening Results

Antibody Pool – Indirect Coombs Test

3 Cell Panel -

	Rh-Hr	Kell	Duffy	Kidd	Lewis	P	MN	Luth	Xg	IS	37°C	AHG	CC
Donor													
R1wR1													
R2R2													
rr													
RESULTS													

11 Cell Panel -

	Rh-Hr	Kell	Duffy	Kidd	Lewis	P	MN	Luth	Xg	IS	37°C	AHG	CC
Donor													
R1R1													
R1wR1													
R2R2													
Ror													
r'r													
r''r													
rr													
rr													
rr													
rr													
R1R1													
RESULTS													

Name

Serial Number

Blood group

Antibody Identified

Signature of Candidate

Signature of Supervisor

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o..... .....am willing to participate in the study **"TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS"**

The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

Name:

.....
Signature of the patient

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____

गवाह के हस्ताक्षर

नाम:

_____

रोगी के हस्ताक्षर/अंगूठे का निशान

नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫ਼ਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"TO STUDY THE FREQUENCY AND PATTERN OF IRREGULAR RBC ALLOANTIBODIES IN BLOOD DONORS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ